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END

Untersuchung über das ätherische Öl aus Podocarpus macrophylla, Don.

II. Mitteilung. Über die neuen Diterpen (α–Podocarpren und β–Podocarpren).

Von

Kitsuji NISHIDA und Hidetaka UOTA.

(Eingegangen am 10 Dez. 1930)

Um die Bestandteile der hochsiedenden Fraktionen zu erklaren, die Elementalanalyse wurde ausgeführt. Die folgenden Ergebnisse wurde erhalten.

Fraktion	Material	CO_2	H_2O	C %	H %	O %
VI	0,1820 g.	0,5530 g	0,1855 g.	82,86	11,40	5,75
VII	5,639 mg	18,356 mg	6,466 mg.	88,77	12,83	0
VIII	4,263 〃	13,179 〃	4,734 〃	84,31	12,42	3,27
IX	6,689 〃	20,766 〃	6,979 〃	84,66	11,67	4,67
X	5,778 〃	18,130 〃	6,151 〃	85,57	11,91	2,52
XI	0,1474 g	0,4744 g.	0,1571 g.	87,77	11,93	0,30
XII	3,649 mg	18,475 mg	4,075 mg.	88,54	12,49	0
IV′	0,2007 g	0,607 g	0,1929 g.	82,71	10,75	6,54
VI′	5,726 mg	18,126 mg	5,933 mg.	86,33	11,59	1,08
VII′	4,409 〃	14,324 〃	4,999 〃	88,61	12,69	0

Aus diesen Ergebnisse geht also hervor, dass die Fraktion VI, VIII, IX, X, IV′ und VI′ sauerstoffhaltig sind und Fraktion XI, XII, VII′, VIII′ und IX′ aus sauerstoffireien Bestandteilen bestehen. Aus Fraktion IX′, X′ und XIII ergaben die nadelformige Kristalle. Jetzt versuchen wir noch eine Weiterfraktionierung (sauerstoffunhaltige Anteile mit Natrium) zur glatte Trennung der einheitlichen Terpenen.

Fraktion	Druck	Temperatur	Farbe	d_{15}^{4}	$[n]_D^{20}$	$[\alpha]_D^{20}$	Mol.-Refr
VII-a	8,5–9,5mm	114∼119°	hellgelb	0,9038	1,492950	−7°,60	65,65
VII-b	12 mm.	126∼132°(129°)	〃	0,9051	1,495582	−6°,00	65,85
VIII-a	9 〃	122∼130°(130°)	〃	0,9168	1,497082	——	65,18
VIII-b	9 〃	150∼170°	hellbraun	0,	1,507730	——	——
XI-a	8 〃	122∼125°	hellgelb	0,9062	1,498012	−4°,28	66,05
XI-b	8 〃	128∼135°	farblose	——	1,501698	——	——
XI-c	9 〃	140∼165°	〃 *	0,9405	1,507730	——	86,25

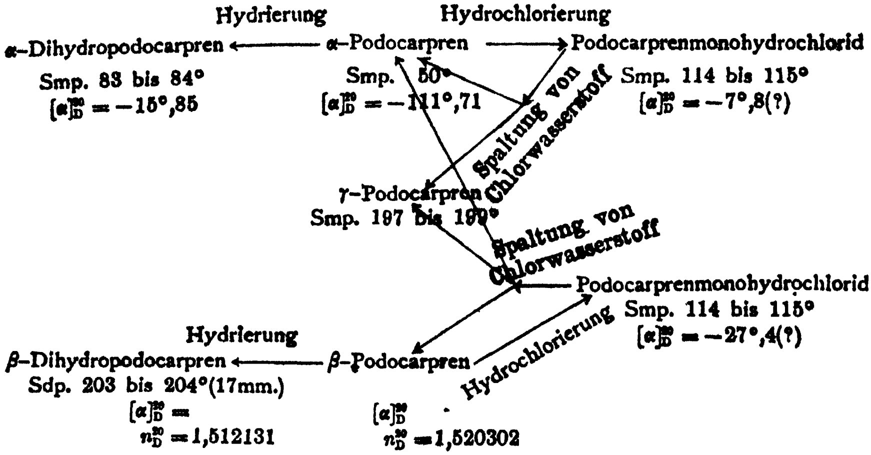
XI-d	12 mm	178~190°	farblose	0,9596	1,515760	− 4°,12	85,66
XII-a	10 〃	165~175°	〃	0,9462	1,512398	——	86,40
XII-a′	11 〃	184~187°	〃	0,9609	1,518390	− 3°,24	85,91
XII-b	9,5 〃	178~180°	〃	0,9632	1,519260	− 4°,36	85,83
XII-b′	10 〃	185~187°	〃	0,9650	1,520652	−10°,44	85,86
XII-b″	12 〃	192~193°	〃	0,9707	1,521678	−18°,20	85,50
XII-c	9 〃	188~190°	〃	0,9688	1,520302	−15°,88	85,48
XII-d	9 〃	195~200°	〃	0,9688	1,521936	−22°,60	85,70
XIII-a	11 〃	193~195°	〃	0,9661	1,522280	−30°,92	85,99
VI′-a	7 〃	ca. 120~140°	〃	0,9834	1,502802	− 6°,40	81,81
VI′-b	7 〃	ca. 165~170°	〃	0,9619	1,514528	−13°,72	85,28
VII′-a	7 〃	165~167°	〃	0,9619	1,511567	−13°,64	85,36
VII′-b	5 〃	173~175°	〃	0,9643	1,518912	−17°,40	85,68
VIII′-a	10 〃	183~187°	〃	0,9664	1,521334	−24°,40	85,83
VIII′-b	10 〃	190~195°	〃	0,9659	1,522538	−29°,88	86,04
IX′-a	9 〃	182~188°	〃	——	1,520560	——	——
IX′-b	9 〃	189~190°	〃	0,9663	1,521162	−26°,20	85,82
IX′-c	11 〃	197~198°	〃	0,9743	1,522965	−37°,80	85,36

Aus der Molekularbestimmung und Mol.-Refr. der Fraktion VII-a, VII-b, VIII-a, VIII-b und X-a ergibt sich der Gehalt an Sesquiterpen und Sesquiterpenalkohol.

Die Krystall aus Fraktion XIII, IX′, und X′ schmolzen bei 50°; $[\alpha]_D^{20}$ betrug-111°,71 in 9,84% iger Chloroformlosung, Molekulargewicht wurde durch Gefrierpunktserniedrigung der bromoformigen Losung ausgefuhrt, und die Zahl war 271 und 270 ($C_{20}H_{32}=272$). Mit Chlorwasserstoff entstand ein Momohydrochlorid $C_{20}H_{32}\cdot HCl$ mit dem Smp. 114 bis 115°, die durch Abspaltung von Halogenwasserstoff in den zwei Krystallen, der eine bei 48 bis 55°, der andere bei 197 bis 199° schmolzen, zuruckgeführt wurde.

Bei der Hydrierung mit Platinschwarz als Katalysator erhielt Dihydroditerpen (Smp. 83 bis 84°, $[\alpha]_D^{20}$, −15°,85). Wir haben diesen ursprünglichen Krystall (Smp. 50°) α-Podocarpren und den durch Abspaltung von Chlorwasserstoff gewonnenen Krystall (Smp. 197 bis 199°) γ-Podocarpren genannt.

Neben α-Podocarpren enthalten die hochstsiedenden Anteile noch eine zweites Diterpen, das β-Podocarpren. Sdp. 188° bis 190° (9mm), d_{15}^{D} 0,9688, $[\alpha]_D^{20}$ −15,88 n_D^{20} 1,520302; Mol.-Refr. 85,48, berechnet fur $C_{20}H_{32}$ 85,29. Das Hydrochlorid dieses Diterpens schmolz bei 114 bis 115°. Durch Abspaltung von Halogenwasserstoff wurde es in drei Isomeren wie folgende Schema verwandelt. Durch der Hydrierung lieferte es Dihydroditerpen, Sdp. 203 bis 204° (17mm.), $[\alpha]_D^{20}$ +7,80, n_D^{20} 1,512131.



Chemische Untersuchungen uber das Glukomannan aus "Konjak".

III. Mitteilung. Acetylierung des Glukomannans.

Von

Kituji NISHIDA und Hideo HASHIMA.

(Aus dem Forstchem Institut, Kiushu Kaiserl Universitat)

(Eingegangen am 10 Dez 1930)

Zur Acetylierung des Glukomannans verwendeten wir Essigsaureanhydrid bei Gegenwart von Pyridin, Zinnchlorid und Zinkchlorid. Die folgende Tabelle enthalt die Werte der nach verschiedener Acetylierung erhaltenen Produkte:

Katalysator	Schmelzpunkt	Essigsäure
Pyridin	218∼226°C	31,50%
Zinnchlorid	210∼220°C	59,79∼64,20%
Zinkchlorid	180∼190°C	62,68%

Wenn Pyridin als Katalysator verwendet wurde, so war das entstehenden Produkt hauptsächlich ein Glukomannomonacetat (Essigsaure 29,41%). Wenn mit Zinnchlorid und Zinkchlorid acetyliert wurde, so enthielt es im wesentlichen ein Triacetat (Essigsaure 62, 50%). Die Schmelzpunkte der Ester waren sehr unscharf und weit schwankender. Wenn man ein Gemisch von Chlor und Schwefeldioxyd benutzt, so erhalt man Ester, die in ihrer Grundzusammensetzung nur sehr wenig verandert sind. In einem Falle wurden die Mischung von Eisessig und Essigsaureanhydrid, das 0,84% Chlor und 6,08% Schwefeldioxyd enthielt, auf dem Wasserbade erhitzt. Es wurde allmahlich gelatinos und nach 5 Minuten langer Erhitzung wurde Alkohol hinzugefügt, dann der Ester mit Chloroform und mit Petroleumather als ein weisser, feiner Niederschlag abgeschieden. Das Produkt enthielt 60,77% Essigsaure und hatte einen Schmelzpunkt von 170~187°C. Wenn Essigsaureanhydrid anstatt Eisessig verwendet wurde, der Prozentsatz von Chlor und Schwefeldioxyd verschieden war und bei Zimmertemperatur 1~3 Stunden lang geschüttelt wurde, erhielt man fast die gleichen Produkte:

	Essigsäure	Schmelzpunkt
Verfahren-B	63,65%	185~194°C
〃 -B	60,33%	188~195°C
〃 -C	63,30%	176~187°C

Es wurde weiter versucht, die Triester moglichst einheitlich durch Ausscheidung mit Chloroform und Petroleumather zu trennen. Durch mehrmaliges Umlosen liess sich der Schmelzpunkt auf 187°C bis 190°C bringen, dabei 174~180°C fing es zu sintern an. Es ist wenig niedriger als bei Fruktoglukomannan (197~221°C). Die Praparate losen sich leicht in Chloroform mit Alkohol, Aceton, Bromoform, aber nicht. in Wasser, Aether, Alkohol, Benzol und Petroleumather.

Das spezifische optische Drehungsvermogen des Esters (in 1 : 9 Alkohol -Chloroform) ist $[\alpha]_D^{15} = -21°,5$.

Triacetylester wurde vakuumtrocknen analysiert und gab folgende Zahlen:

	Gefunden		Berechnet für
	I	II	$[C_6H_7O_2\cdot(OCOCH_3)_3]_x$
C	50,01	50,30	49,98
H	6,32	6,79	5,60

Als Molekulargewicht fanden wir, durch Gefrierpunktserniedrigung der bromoformigen Losung, die Zahlen 2299 und 2383; aus den Formeln $[C_6H_7O_2$

$(OCOCH_3)_3]_9$ und $[C_6H_7O_2(OCOCH_3)_3]_6$ berechnen sich die Molekulargewichte 2592 und 1782. Die Wahrscheinlichkeit spricht für die Formel $[C_6H_7O_2(OCOCH_3)_3]_9$.

Chemische Untersuchungen über das Glukomannan aus "Konjak".

IV. Mitteilung. Acetolyse des Glukomannans.

Von

Kituji NISHIDA und Hideo HASHIMA

(Eingegangen am 9 Mar. 1931)

Aus den Ergebnissen der Hydrolyse mit Salz- und Schwefelsaure geht also hervor, dass das Glukomannan ein Mannose- und Glukosederivat (2 : 1) ist, darüber hinaus haben wir eine Reihe neuartiger Umsetzungsprodukte durch Acetolyse zu Tage fordern konnen. Aus dieser Klarstellung kommen wir der Frage von Stellung der Mannose und der Glukose in dem Glukomannan bedeutend naher.

Wir haben die Acetolyse des Glukomannans in der Warme und in der Kalte mit einem Gemisch aus Essigsaureanhydrid und konz. Schwefelsaure nach Skraup und Koenig ausgefuhrt. Dabei fiel kein flockiges Produkt aus, das war wahrscheinlich in der Hitze über das Zwischenprodukt zum Hexoseacetat abgebaut worden. Wir haben uns bemüht diese Umsetzung so zu vermindern und durch geeignete Versuchsbedingungen die Acetolyse so zu leiten, dass eine moglichst gute Ausbeute des Zwischenproduktes gewonnen wurde. Mit Essigsaureanhydrid, Eisessig und konz. Schwefelsaure in der Kalte war die Ausbeute eine bessere und die Reinigung des erhaltenen Produktes leichter durchführbar.

Aus den verschiedenen Experimentellen geht die Ausbeute je nach den Variationen der Versuchsbedingungen hervor, sie betrug von 98% bis 133%. Die maximale Ausbeute an Rohacetat geht aus diesen Versuchen mit ca. 67 % d. Theorie, entsprechend ca. 72% d. Theorie für absolutes trockenes Glukomannan, hervor. Das folgende Verhaltnis der Reagentien wurde als zwechmassig gefunden.

8 g. Glukomannan, 40 g. Essigsaureanhydrid, 46 g. Eisessig, 4 g. konz. Schwefelsaure,

Die Reaktionsdauer bei Zimmertemperatur (22～26,°5 C) oder in dem Schrank (23°C) wurde mit etwa 15～20 Tagen als die günstigste gefunden.

Das Rohacetat war hauptsachlich in kaltem Alkohol loslich, aber ein Teil war unlöslich. Versuche zur Trennung und Reinigung des Rohacetates wurden in dieser Hinsicht (Loslichkeit) durchgefuhrt, alle Bemuhungen seiner Isolierung aber blieben erfolglos. Die Schmelzpunkt schwankte also zwischen 88～95° C, bei dem unloslichen Teil erhohte er sich auf 131～140°C bis 145～149°C. und war etwas schwankend. Das Produkt fing zuerst an zu sintern, bei 3～5° unter dem Endpunkte, ging es hauptsachlich in die Schmelze uber und wurde schliesslich unter Verfarbung zersetzt. Die Essigsauregehalt schwankte zwischen 65,04% und 70,64% im loslichen Teil, zwischen 62,26% und 63,61% im unloslichen Teil. Es ist bemerkenswert, dass in dem Alkohol unloslichen Teil, d. h. in dem durch der Acetolyse bei geringer Reaktionsdauer (8～10) Tagen) gewonnenen Produkte der Essigsauregehalt ein niedriger ist. Es bedeutet daher diese Schwankung zunachst einen Beweis dafur, dass in diesen Produkten ein hochmolekulares Acetat vorhanden ist. Bei der ersten Einwirkung des Acetolysemittels auf das Glukomannan hat die Veresterung zu Acetylglukomannan stattgefunden, dann wurde es über hochmolekulare Acetate zu Glukose- und Mannosepentaacetat umgewandelt, wie das folgende Schema zeigt.

Glukomannan → Triacetylglukomannan (Essigsaure 62,50%) → Hochmolekulare Acetate des Glukomannans (Zwischenprodukt) → Hendekaacetylglukomannotrihexose (Essigsaure 68,29%) → Octaacetylmannobiose oder Octaacetylglukomannobiose (Essigsaure 70,80%) Pentaacetylmannose und -glukose (Essigsaure 76,92%).

Das Acetat loste sich in weniger kaltem Alkohol nach vielmaligem Ausziehen mit heissem Wasser teilweise auf. Der zuruckbleibende Sirup gab nach mehrmaligem Umfallen die Kristalle von Hendekaacetylglukomannotrihexose, die 69,78% Essigsaure enthalten, und bei denen $[\alpha]_D^{20}$ 15°,1 ist. Sie besitzen weisse Wetzsteinform, fangen bei 90～93°C. an zu sintern und schmelzen bei 108～110°C. unter Braunung und Zersetzung. Die Elementaranalyse erbrachte den Beweis dafür, dass es sich bei den Kristallen um Hendekaacetylglukomannotrihexose handelt;

	Gefunden		Berechnet für
	I	II	$C_{40}H_{54}O_{27}$
C	49,65%	49,38%	49,67%
H	5,72%	5,77%	5,63%

Das Molekulargewicht wurde durch Gefrierpunktserniedrigung der benzoligen Lösung ausgeführt, und die Zahl war 924 und 1014; aus den Formeln [$C_{18}H_{21}O_5(OCOCH_3)_{11}$] und [$C_{12}H_{14}O_3(OCOCH_3)_8$] berechnet sich das Molekulargewicht auf 966 und 678. Da das gefundene Molekulargewicht ein wenig zur Hendekaacetylglukomannotrihexose überstimmte, ist anzunehmen, dass die Kristalle wahrscheinlich noch kleine Mengen nieder– oder hochmolekularen Acetate enthielten.

Wenn auch die Loslichkeit der Acetate nur wenig Unterschiede zeigt, so ist doch die Durchfuhrung der Trennung sehr schwierig. Viel besser kommt man zum Ziel, wenn man mit Aether bis zur Erschopfung auszieht. Bei standiger Extrahierung klebte das Acetat als klumpige Masse zusammen, diese wurde herausgenommen, dann pulverisiert, wieder von neuem mit Aether ausgezogen. Es wurde nach mehrmaligem Ausziehen mit Alkohol als der Kristall erhalten. Aus diesen Versuchen geht also hervor, dass der atherlosliche Teil viele Acetylgruppen aufnimmt und zwar rund 70,7%, darin wahrscheinlich Hexoseacetat vorhanden ist. Der Schmelzpunkt von dem atherloslichen Acetate war niedriger als der des atherunloslichen Teiles. Das Drehungsvermogen war in Chloroform erhoht und nach der Ausziehung mit Aether zeigte das Produkt ein hoheres spezifisches Drehungsvermogen. Das gefundene Molekulargewicht stimmt an besten mit der Formel $C_{18}H_{21}O_5(OCOCH_3)_{11}$ uberein.

Eine Reihe von Versuchen der Trennung wurde weiter fortgefuhrt, wobei Pentaacetylhexose durch die wiederholte Ausziehung mit heissem Wasser abgetrennt wurde. Schliesslich hat die Abtrennung der Einheiten durch viermalige fraktionierte Ausscheidung mit 50% igem Methylalkohol nur ergeben, dass die Hendekaacetylglukomonnotrihexose loslich ist, und durch langsames Abkühlen auf 5∼0°C. isolieren werden kann. Schmelzpunkt: 95∼110°C., $[\alpha]^{20}_{D}$ in Chloroform + 18°, in Benzol + 6°. Es lost sich in Chloroform, Bromoform, Methyl– und Aethylalkohol, ziemlich schwer in Aether und nicht in Wasser und Petroleumather.

Die Verseifung wurde mit Barytwasser unter fortwahrendem Schütteln bei 30°C. ausgeführt. Nach etwa 6 Stunden wurde das Baryt mit Schwefelsaure quantitativ entfernt und das Filtrat unter vermindertem Druck eingeengt. Nach langem Stehen im Exsiccator stellte sich keine Ausscheidung von Kristallen der Glukomannotrihexose ein, die aber bei Zusatz von Alkohol als weisses Pulver ausfiel, in dem noch ein Spur Barium zuruckgeblieben war. Schmelzpunkt: 118∼120°C. unter Sintern, etwa 175°C. unter starkem Aufschaumen, 182°C. unter Zersetzung mit Braunverfarbung.

Die Darstellung der reinen Glukomannotrihexose aus Acetat gelang nur mit absolutem methylalkoholischem Ammoniak, in dem das Acetat wurde bei 0°C. ausgelöst. Die Mischung wurde 20 Stunden lang im Eisschrank aufbewahrt, dann im Vakuum eingedampft und mit absolutem Methylalkohol ausgezogen. Der Methylalkoholauszug kristallisierte nach dem Zusatz von Aether am Wande der Schale. Die erhaltene Glukomannotrihexose zeigte unter dem Mikroskope die in nadelige Form.

Nach mehrmaligem Umkristalliesieren aus Methylalkohol und Aether zeigte die Glukomannotrihexose den Schmelzpunkt 216,5~217°C., unter Sintern von 206°C., $[\alpha]_D^{20}$ in Wasser $-16{,}^\circ 4$. Die Mikroelementaranalyse ergab.

	Gefunden:		Berechnet für: $C_{18}H_{32}O_{16}$
C	42,38%	42,64%	42,83%
H	6,94%	9,94%	6,40%

Das Molekulargewicht fand sich durch Gefrierpunktserniedrigung der wässerigen Lösung, welche Zahl 513 betrug, entsprechend der Formel $C_{18}H_{32}O_{16} = 504$. Der Mutterlaugerückstand nach Zusatz von Aether wurde als Glukomannotrihexose abgeschieden, davon ein Anteil als nadelige Kristalle und andere Anteile als weisses Pulver dargestellt wurden. Der Schmelzpunkt der Kristalle war gleich gross wie der Glukomannotrihexose.

Die Spaltung der Glukomannotrihexose mit 2 %iger Schwefelsäure hat ergeben, dass der Bestandteil der Glukomannotrihexose als Mannose- und Glukosederivate (2 : 1) in vollkommen gleicher Menge wie der des Glukomannans vorhanden ist. Diese Ergebnis ist der Schlüssel zu dem Verhältnis der Glukomannankonstitution geworden.

Hier war die Acetolyse schon über dem für Hendekaacetylglukomannotrihexose charakteristischen Zustand hinausgegangen und es waren darüber die zu sehr weit abgebauten Stoffe von kleinerem Molekulargewicht z. B. Bioseacetat, Hexoseacetat, abgespalten. Dabei konnte man zwei Arten Disaccharide gewinnen.

Disaccharid (A) war ein Mikrokristall, Schmelzpunkt 150~160°C., spezifische Drehung $[\alpha]_D^{20}$ $+10{,}^\circ 5$, Das Molekulargewicht fand sich durch Gefrierpunktserniedrigung mit den Zahlen 296 und 375, die der Formel $C_{12}H_{20}O_{11} = 342$ entsprechen. Durch Hydrolyse mit 2 % iger Schwefelsäure lieferte es ein Mol Mannose und ein Mol Glukose.

Disaccharid (B) gab ein warzenförmiges Kristall, das die folgenden Konstanten aufwies: $[\alpha]_D^{20}$ in Wasser $-7{,}^\circ 9$, Molekulargewicht $= 324$. Der Schmelzpunkt war unscharf und lag 135~140°C. Es liess sich in zwei Mol Mannose hydrolysieren, war also eine Mannobiose. Hierdurch waren die Präparate (A) und (B) nicht einheitlich, aber es liess sich erfassen, dass die Acetolyse mit Essigsäureanhydrid und Schwefelsäure über Trisaccharid- und

2 Arten Disaccharidacetat bis zu dem Mannose- und Glukoseacetat führt, daraus muss man sich das Glukomannanmolekül aus 2 Mol Manneseresten und 1 Mol Glukoserest, wie folgende Schema zeigt, aufgebaut denken.

$$\text{Glukomannan [2 Mol Mannose + 1 Mol Glukose]}_x \xrightarrow{\text{Acetolyse}} \underset{[\alpha]_D^{20} = -18^\circ}{\overset{\text{Smp. } 95\sim110^\circ\text{C.}}{\text{Hendekaacetylglukomannotrihexose}}}$$

$$\longrightarrow \text{[2 Mol Mannose + 1 Mol Glukose]} \xrightarrow{\text{Verseifung}} \underset{[\alpha]_D^{20} = -16^\circ}{\overset{\text{Smp. } 217^\circ\text{C.}}{\text{Glukomannotrihexose}}} \longrightarrow$$

$$\longrightarrow \text{[2 Mol Mannose + 1 Mol Glukose] (Laevidulin oder Laevidulinose)} \longrightarrow$$

$$\text{Disaccharide} \left\{ \begin{array}{ll} \text{(A)} & \begin{array}{l} \text{Smp. } 150\sim160^\circ\text{C.} \\ \text{Glukomannobiose} \\ [\alpha]_D^{20} = 10,^\circ 5 \end{array} \\ \text{(B)} & \begin{array}{l} \text{Smp } 125\sim140^\circ\text{C.} \\ \text{Mannobiose} \\ [\alpha]_D^{20} = -7.^\circ 9 \end{array} \end{array} \right. \longrightarrow \left\{ \begin{array}{l} \text{Mannose} \\ \\ \\ \text{Glukose} \end{array} \right.$$

Über das Vorkommen von Lyso-Lecithin in poliertem Reis.

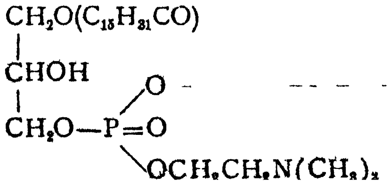
Von

Motoe IWATA.

(*Mitgeteilt von U. Suzuki, Biochemisches Laboratorium des Instituts für physikalische und chemische Forschung, Tokio.*)

(Eingegangen am 9. Mar 1931)

Wenn polierter Reis mit Wasser gewaschen, getrocknet und wiederholt mit warmem 94 % igem Alkohol extrahiert und der alkoholische Extrakt im Vakuum eingedampft wird, so erhalt man einen hellbraunen Sirup, welcher etwa 1% des Ausgangsmaterials betragt. Der Verfasser hat nun aus diesem alkoholischen Extrakt, ausser Neutralfetten und Fettsauren, wie Palmitin-, Olein- und Linolsauren, eine stark hamolytisch wirkende Substanz isoliert und als Lyso-Lecithin identifiziert ·



Lyso-Lecithin

Nach Delezenne(1), Belfanti(2) und Magistris(3) ist Lyso-Lecithin ein Gift, welches durch Einwirkung von Kobragift auf Lecithin entsteht. Das Kobragift selbst enthält kein Gift, sondern es enthalt ein wirksames Enzym "Lecithinase", welches aus Lecithin ein Molekül ungesattigter Fettsaure abspaltet und das giftige Lyso-Lecithin liefert.

Es ist eine auffallende Tatsache, dass der Reis eine solche giftige Substanz enthält. Ob es mit dem hypothetischen "Oryzatoxin" von Teruuchi und seinen Mitarbeitern(4) identisch ist, oder ob es irgendeine Beziehung mit der Beriberikrankheit des Menschen hat, wie diese Autoren behaupten, muss spater untersucht werden.

Isolierung des Lyso-Lecithins.

Zu diesem Zwecke wurde der alkoholische Extrakt unter vermindertem Druck stark eingeengt und in eine grossere Menge Äther eingegossen. Der dadurch entstandene Niederschlag wurde in kaltem, absolutem Alkohol gelöst, vom unlöslichen Ruckstand abfiltriert und wiederum in Äther eingegossen. Der weisse Niederschlag wurde abgesaugt und im Exsikkator getrocknet. Die Ausbeute betrug 150 g. aus 70 kg. Reis, was etwa 0,2% des Ausgangsmaterials entspricht.

Dieser Niederschlag wurde nun wieder in warmem, absolutem Alkohol gelöst. Nach dem Erkalten vom unloslichen Rückstand abfiltriert und mit einer warmen alkoholischen Losung von Cadmiumchlorid versetzt, bis es keine Fallung mehr gab. Der Cadmiumniederschlag wurde wiederholt mit absolutem Alkohol gewaschen, in 10- bis 15 fachen Mengen heissem Chloroform suspendiert und mit kleinem Überschuss von 95% igem Alkohol, welcher 15% Ammoniak enthielt, versetzt. Es schieden sich dabei Cadmiumhydroxyd und Ammoniumchlorid als weisser Niederschlag aus. Man filtrierte nun ab und dampfte das Filtrat unter vermindertem Druck ein ; so wurde das rohe Lyso-Lecithin in kristallinischem Zustande erhalten.

Reinigung des Lyso-Lecithins.

Die in oben erwahnter Weise dargestellten Kristalle von Lyso-Lecithin waren sehr hygroskopisch und enthielten noch verschiedene Substanzen als Verunreinigung ; sie wurden deshalb wiederholt aus absolutem Alkohol und

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(1) Delezenne und Ledebt: C. r. Acad. Sci., **152**, 790, (1910); **153**, 81, (1911); **155**, 1101, (1912); Delezenne und Fourneau: Bull. Soc. Chim., IV, **15**, 421, (1914).

(2) S. Belfanti: Biochem. Zeitschr., **154**, 148, (1924); Zeitschr. f. Immunitätsforschung, **44**, H. 4/5, (1924); **56**, H. 5/6, (1928).

(3) H. Magistris: Biochem. Zeitschr., **210**, 85 bis 119, (1929).

(4) M. Teruuchi: Keio-Igaku, **9**, Nr. 11, (1929).

Chloroform umkristallisiert, um Chlor und Ammoniak vollstandig zu entfernen, dann wurde es nochmals in fünffacher Menge warmen Pyridins gelost und im Exsikkator stehengelassen. Es schied sich dabei das Lyso-Lecithin in feinen seidenglanzenden Nadeln aus. Dieser Prozess wurde dreimal wiederholt und schliesslich die Kristallmasse in absolutem Alkohol gelost, mit wenig Aceton versetzt und stehengelassen. Das Lyso-Lecithin schied sich in Form farbloser langer Nadeln aus, die gesammenlt und über Chlorcalcium und dann über konzentrierter Schwefelsaure im Exsikkator getrocknet wurden.

Das reine Lyso-Lecithin bildet farblose Nadeln, in geschlossenem Rohrchen erhitzt, zersetzt es sich bei 262 bis 264° unter Gasentwicklung. Es ist loslich in Wasser, Äthyl- und Methylalkohol, Eisessig, Chloroform und Pyridin, aber unloslich in Ather, Petrolather und Aceton. Die wasserige Losung reagiert gegen Phenolphthalein neutral und hat stark stechenden Geschmack. Wie organische Basen wird es durch Phosphorwolframsaure gefallt. Wenn eine wasserige Losung des Lyso-Lecithins mit Bariumhydroxyd erwarmt wird, so wird es leicht hydrolysiert und gibt dabei weisse Trübung. Es gibt keine Reaktion der ungesattigten Fettsauren mit konzentrierter Schwefelsaure und Zucker. Ferner gibt es keine Molischsche Reaktion auf Zucker. Mit Cadmiumchlorid oder Platinchlorid bildet es nadelformige Kristalle.

Bestimmung des Drehungsvermögens.

1. 1,066 g. Lyso-Lecithin in 25 ccm. Chloroform gelost, spezifisches Gewicht der Losung 1,476. Drehung bei 20° und Natriumlicht, im 2-dcm-Rohr 0,4° nach links. Mithin

$$[\alpha]_D^{20} = -4,52^\circ.$$

Wenn man 0,02 bis 0,1 g. Lyso-Lecithin in Wasser lost und Tauben subkutan einspritzt, so beobachtet man lokale Verblutung und starke Diarrhoe nach 3 bis 4 Stunden. Der Kot ist grunlich gefarbt. Wird dieselbe Dosis einer Maus eingespritzt, so stirbt sie nach 3 bis 12 Stunden. Wenn es aber per os gegeben wird, wirkt es weniger giftig. Wird 0,1 g. Kristall in 1 Liter Wasser gelost und ein kleiner Goldfisch (15 bis 20 g.) hineingebracht, so scheidet der Fisch schleimige Substanz aus dem Munde und der ganzen Körperoberflache aus und stirbt innerhalb 1/2 bis 1 Stunde.

Analyse des Lyso-Lecithins.

Präparat I. Viermal aus Pyridin und schliesslich aus heissem Chloroform umkristallisiert, über konzentrierter Schwefelsaure und Phosphorpentoxyd im Exsikkator getrocknet und analysiert.

1.	0,2299 g.	Substanz:	0,4895 g. CO_2,	0,2083 g. H_2O.
2.	0,2151 g.	"	0.4590 g. CO_2.	0,1917 g. H_2O.
3.	0,2578 g.	"	nach Kjeldahl 2,73% N	
4.	0,2517 g.	"	" " 2,53% N.	
5.	0,2537 g.	"	0,0540 g. $Mg_2P_2O_7$ = 5,95% P.	
6.	0,2492 g.	"	0,0534 g. $Mg_2P_2O_7$ = 5,97% P.	

Präparat II. Aus heissem, absolutem Alkohol durch Zusatz von wenig Aceton umkristallisiert, über Chlorcalcium und konzentrierter Schwefelsaure im Exsikkator und schliesslich im Vakuumtrockenapparat getrocknet und analysiert.

1.	0,1821 g.	Substanz:	0,3873 g. CO_2,	0,1586 g. H_2O.
2.	0,1841 g.	"	0,3905 g. CO_2,	0,1670 g. H_2O.
3.	0,1785 g.	"	0,3802 g CO_2,	0,1650 g H_2O.
4.	0,1973 g.	"	nach Kjeldahl 2,61% N.	
5.	0,2338 g.	"	0,0527 g. $Mg_2P_2O_7$ =-6,28% P.	
6.	0,1920 g	"	0,0427 g $Mg_2P_2O_7$ = 6,19% P	

	C	H	N	P	O
Durchschnittswerte der Analysen	58,08	10,01	2,62	6,24	23,05
Berechnet für $C_{24}H_{50}NPO_8$	56,32	9,85	2,74	6,07	25,02
" " $C_{24}H_{50}NPO_7$	58,18	10,10	2,84	6,26	22,63

Hydrolyse des Lyso-Lecithins.

1. *Palmitinsäure.* Zur Isolierung der Palmitinsaure wurden 1,10 g. Lyso-Lecithin in 350 ccm. 10% iger Schwefelsaure gelost und im Soxhletschen Apparat 24 Stunden mit warmem Äther extrahiert. Die atherische Losung wurde wiederholt mit Wasser gewaschen, um Schwefelsaure zu entfernen, und mit Natriumsulfat getrocknet. Nach dem Eindampfen des Äthers schied sich die Palmitinsaure in weissen Kristallen aus. Die Ausbeute betrug 0,541 g. oder 97% der Theorie. Schmelzpunkt des gereinigten Praparats 62,5°. Mischprobe mit reiner kauflicher Palmitinsaure ergab keine Erniedrigung des Schmelzpunktes. Saurezahl: 218,6

2. *Cholin.* Das Cholin wurde aus der wasserigen Losung, nach der Extraktion der Palmitinsaure, mit Phosphorwolframsaure gefallt. Der Niederschlag wurde in gewohnlicher Weise durch Baryt zerlegt und der überschüssige Baryt durch Kohlensaure entfernt. Das Filtrat wurde stark eingeengt und mit absolutem Alkohol versetzt. Cholincarbonat schied sich als farbloser Sirup aus. Ausbeute: 0,265 g. oder 24% des Lyso-Lecithins (theoretisch: 24,65%).

Cholincarbonat wurde zum Pikrat verwandelt, mit einem Gemisch von Alkohol und Äther gewaschen und aus heissem Alkohol umkristallisiert.

Gelbe Prismen; Schmelzpunkt 244° (unkorr). Mit reinem Cholinpikrat gemischt, zeigte der Schmelzpunkt keine Erniedrigung.

3. *Glyceroplosphorsäure.* Das Filtrat vom Phosphorwolframsaureniederschlag wurde mit Baryt neutralisiert, abfiltriert, zu einem Sirup eingedampft und in eine grössere Menge Alkohol eingegossen. Es entstand ein weisser Niederschlag, welcher in kaltem Wasser leicht, in heissem Wasser schwer loslich war. Er gab keine Reaktion auf Zucker und enthielt Phosphor und Barium, aber keinen Stickstoff; so wurde es als Bariumsalz der Glycerophosphorsaure identifiziert. Wegen Mangel an Material wurde es nicht analysiert.

Über die chemischen Beschtendteile des Tabaks.

1. Mitteilung. Uber die organischen Basen und Sauren der frischen Tabakblatter.

Von

Kazuo YAMAFUJI.

(Aus dem Agrikulturchem Laboratrium der Landw. Abteilg. der Kyushu Kaiserl. Univ.)

(Eingegangen am 27. Dezember 1930)

A. Die organischen Basen.

3kg. frisch gepflückter Tabakblatter wurden im Wasserdampfschrank 1/2 Stunde erhitzt und dann unter starken Sonnenstrahlen schnell getrocknet. Die qauntitative Bestimmung ergab (nach Igaki):

bezogen auf:	Frischgewicht %	Trockensubstanz %
Gesamtstickstoff	0.55	3.26
Eiweissstickstoff.	0.33	2.03
Basenstickstoff.	0.096	0.57
Nitratstickstoff.	0.025	0.15
Nikotin.	0.357	2.12

Die Wasserauszuge dieser Tabakblatter wurden mit Bleiazetat und Bleiessig gefallt, das Filtrat vom Bleiniederschlag mit Schwefelwasserstoff zersetzt und stark eingeengt.

Die Lösung wurde mit Sodalosung alkalisch gemacht und im Wasserdampfstrom destilliert. Aus dem Destillat wurde Nikotin dargestellt.

Nikotinpikrat; Schmelzp. 217~8°C (unkorr.)

73.81% Pikrinsäure. (Ber. f. $CHN(CHON)$ 73,87% Pikrinsäure).

Nikotinchlorplatinat; 24,15% Pt (Ber. f. $CHN2HCl$) $PtCl$ 24,22 % Pt).

Der Destillationsrückstand wurde mit Schwefelsäure angesäuert und mit Phosphorwolframsäure ausgefällt. Die aus dem Phosphorwolframsäureniederschlag in bekannter Weise dargestellte Lösung, die freie Basen enthielt, wurde

1. mit Silbernitrat gefällt, und das Filtrat.
2. mit Silbernitrat und Baryt gefallt.
3. Das Filtrat von Silbernitrat und Baryt-Niederschlag wurde nochmals mit Phosphorwolframsäure gefällt.

Aus Fraktion 1 wurde Adenin erhalten. Adeninpikrat: Schmelzp. 280°C 62.82% Pikrinsäure (Ber. f. $C_5H_5N_5C_6H_3O_7N_3$, 62,91% Pikrinsäure).

Adeninchloraurat; 41,37% Au (Ber f. $C_5H_5N_5 \cdot HCl \cdot AuCl_3$, 41,50% Au).

Fraktion 2 lieferte Histidin und wahrscheinlich Arginin.

Histidindihydrochlorid; Schmelzp. 231°C Diazoreaktion positiv.

30,71% Cl (Ber. f. $C_6H_9O_2N_3 \cdot 2HCl$, 31,14% Cl).

Arginin wurde in genügender Menge nicht erhalten, um die Analyse auszuführen. Diazetylreaktion positiv.

Aus Fraktion 3 wurde Betain gewonnen.

Betainpikrat; Schmelzp. 180°C.

Betainchloraurat; 43,31% Au (Ber. f. $C_5H_{11}O_2N \cdot HCl\ AuCl_3$, 43,13% Au).

Betainchlorplatinat; 30,40% Pt (Ber. f. $(C_5H_{11}O_2NHCl)_2\ PtCl_4$, 30,25% Pt).

B. Die organischen Säuren.

8kg. frisch gepflückter Tabakblatter wurden im Wasserdampfschrank 1/2 Stunde erhitzt und dann schnell getrocknet.

Diese Tabakpulver wurden mit salzsäurehaltigem Wasser ausgekocht, und die so erhaltenen Wasserauszüge mit Bleiazetat und Bleiessig gefallt. Der Bleiniederschlag wurde mit Schwefelwasserstoff zersetzt, die Lösung der Sauren im Vakuum zu einem dicken Sirup eingedampft und dieser zur Ausfallung des Pektinstoffe mit absolutem Weingeist versetzt. Das Filtrat wurde wiederum zu dicken Sirup eingedampft und nochmals mit absolutem Weigeist behandelt. Nach Entfernung der Pektinstoffe wurde so viel kalt gesättigte alkoholische Salzsäure hinzugefügt, dass die Gesamtmenge 3% Chlorwasserstoff enthielt, und 5 Stunden zum Sieden erhitzt. Die Esterlösung wurde im Vakuum zur Sirupdicke eingedampft und der Rückstand mit Aether durchgeschüttelt.

Die ätherische Lösung der Ester wurde sorgfältig Pottaschelösung gewaschen und über Natriumsulfat getrocknet. Dann wurde der Aether abdestilliert und der Esterrückstand im Vakuum der gebrochenen Destillation unterworfen.

1.	30∼35°C	bei 40mm	1.5g.	farblos.
2.	32∼65	〃 30	1 0	〃
3.	65∼85	〃 〃	2.0	〃
4.	85∼105	〃 〃	2.5	〃
5.	105∼125	〃 〃	4.0	〃
6.	125∼130	〃 〃	5 0	〃
7.	135∼155	〃 〃	7 0	gelblich
8.	155∼180	〃 〃	9.0	gelb.
	Ruckstand		3 0	

Das Verhalten gegen Semicarbazidazetat- und gegen Phenylhydrazinazetatlosung zeigte, dass alle Fraktionen keine Ketonsaureester enthielt.

Die Ester wurden in Hydrazide und dann in Benzylidenverbindungen ubergefuhrt. Zum Vergleich wurden noch aus reinen von Merck bezogenen Sauren unter gleichen Umstanden die Ester, Hydrazide und deren Benzyliden verbindungen dargestellt und die Mischprobe ausgeführt.

Fraktion 1 gab keine Hydrazid und Benzylidenverbindung.

In der Fraktion 2 fand sich Essigsaureester.

Benzylidenessigsaurehydrazid; Schmelzp. 136°C (unkorr).

17,10% N (Ber. f. $C_9H_{10}ON_2$, 17,27% N).

Fraktion 3 besteht aus Aepfelsaurediathylester.

Hydrazid; Schmelzp. 177∼8°C. 34,56% N (Ber. f. $C_4H_{10}O_3N_4$, 34,57% N).

Benzylidenverbindung; Schmelzp. 173°C. 16,60% N (Ber. f. $C_{18}H_{18}O_3N_4$, 16,51% N).

Das Filtrat des Aepfelsauredihydrazids lieferte eine geringe Menge einer Benzylidenverbindung vom Schmelzp. 199°C.

Die Fraktionen 4, 5, 6 und 7 bestehen aus fast reinem Aefelsaurediathylester.

Fraktion 8 besteht aus Zytronensauretriathylester, dem Spuren von Aepfelsaureester beigemengt sind.

Hydrazid; Schmelzp. 144∼5°C, 35,94% N (Ber. f. $C_6H_{14}O_4N_6$, 35,90% N).

Benzylidenverbindung; Schmelzp. 226°C, 16,69% N (Ber. f. $C_{27}H_{26}O_4N_6$, 16,87% N).

Das Filtrat vom Bleiniederschlag, nach Entbleien mit Schwefelwasserstoff, wurde mit Schwefelsaure versetzt und nun mit Aether extrahiert. Aus dem atherischen Extrakten wurde zunachst der Aether abdestilliert, der Ruckst-

und mit Wasser verdünnt und die wässerige Lösung erschöpfend mit Benzol extrahiert. Die wässerige Lösung wurde dann im Vakuum eingedampft, dieser verestert und die Ester der gebrochenen Destillation im Vakuum unterworfen.

25~30°C bei 40mm. 1.5g. farblos.

Der Ester besteht aus Essigsaureester.

Bestimmung der organischen Säuren.

100g. derselben Tabakblatter wurden mit Schwefelsäure gemischt und mit Aether extrahiert. Nach 50 Stunden wurde die Extraktion unterbrochen, der Aether abdestilliert, der Rückstand in Wasser gelost und nach Kissling's Verfahren die qauntitative Bestimmung der Sauren durchgeführt:

Aepfelsäure, 1.3%. Zytronensäure, 0.6%. Oxalsäure, 0.09%.

Zusammenfassung.

1. Aus ca. 3kg. frischen Tabakblatter wurden isoliert:

Nikotin,	9.0g.	Adenin (Pikrat),	0.1g.
Histidin (Dihydrochlorid),	0.01g.	Arginin,	?
Betain (Chloraurat),	0.2g		

2. Die durch Blei fallbaren Sauren des Tabaks wurden verestert und ihre Ester der fraktionierten Destillation unterworfen. Zur Kennzeichnung wurden die Ester in Hydrazide bzw. deren Benzylidenverbindungen umgewandelt. Die Ergebnisse zeigten, dass die Sauren der frischen Tabakblatter überwiegend aus Aepfelsaure neben verhaltnismassig viel Zytronensaure und Spüren unbekannter Saure bestanden. Das Filtrat vom Bleiniederschlag gab nur Essigsaure, welche wahrscheinlich vom Bleiazetat kam. Die qauntitative Bestimmung der Sauren nach Kissling's Verfahren ergab das folgende Resultat.

Aepfelsäure, 1.3%. Zytronensäure, 0.6%. Oxalsäure. 0.09%.

Bekanntlich ist in frischen gereiften Tabakblattern Starkemehl in sehr bedeutender Menge enthalten. Wahrend der Trocknung und Fermentation verschwindet der grosste Teil desselben und es findet eine Bildung von organischen Sauren statt.

Daraus lasst sich schliessen, dass der grossere Teil der Oxalsäure, die in getrockneten oder fermentierten Tabakblattern gefunden wird, wahrend der Trocknung bzw. Fermentation entstanden ist.

Enzyme in den Eingeweiden vom Haliotus giganteus Gm.

Von

Kokichi OSHIMA

(Aus dem chemischen Laboratorium im Fischerei-Institut,
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(Eingegangen. am 1. Marz 1931)

In den Eingeweiden (hauptsachlich in der sogenannten "Leber") vom Seeohr (Haliotus giganteus Gm.), einer Muschel, die zahlreich im japanischen Meer lebt und am meisten braune Algen frisst, fand der Autor die folgenden Enzyme:

Alginase (Alginsaure stark spaltendes Enzym) Cellulase (Filtrierpapier als Substrat), Amylase, Gelase (Agar-agar spaltendes Enzym), Saccharase (schwach), Maltase, Lab, Protease (Casein als Substrat, schwach).

Die folgenden Enzyme konnte er nicht finden:

Tyrosinase, Amygdalase, Esterase (Olivenöl und Tributyrin als Substrate.) Die Alginase ist ein neu gefundenes Enzym, das Alginsaure in braunen Algen spalten kann. Darüber schrieb der Autor in anderen Berichten genauer.

Alginase, Cellulase und Gelase werden in Lebewesen, ausser in Mikroorganismen, sehr selten gefunden, was auch zu der Tatsache passt, dass Haliotus am meisten auf Meeres-Algen leben.

Über die Entdeckung eines Alginsäure spaltenden Enzymes und seine Eigenschaften I.

Von

Kokichi OSHIMA.

(Aus dem chemischen Laboratorium im Fischerei-Institut,
. Hokkaido Kaiserliche Universität, Japan).

(Eingegangen am 1. Marz 1931)

Alginsäure ist eine Kohlenhydrat-Säure, die reichlich in braunen Algen (20～40% von trockener Substanz) gefunden wird. Der Autor konnte sie reinigen durch Auflösung brauner Algen (Laminaria angustata Kjellm) in 0.5

% Na_2CO_3, filtrierte sie durch ein Tuch und durch Filtrierpapier-Brei. Aus dem klaren Filtrat scheidet sich nach Zusatz von verd. HCl die Alginsaure aus, der Niederschlag wird nochmals durch ein Tuch filtriert.

Obige Ausführung wird 3 mal wiederholt und zuletzt mit Alkohol und Aether gewaschen.

Die Wasserauszuge aus den Eingeweiden (hauptsachlich aus der sogenannten "Leber") des Seeohrs (Haliotus giganteus Gm.) und des Seeigels (Sphaerechinus pulcherrimus Ag.) konnten stark die Alginsaure spalten. Der Autor prufte es durch die Viscositatsanderung der Alginsaurelosung und durch die Reduktionsfahigkeitszunahme der Fehlingschen Losung, und die optimale Wasserstoffionkonzentration von der Wirksamkeit der Viscositatsanderung war pH 7.2. Der Autor hat dieses Enzym als Alginase bezeichnet.

Aber durch die Auszuge aus Schweine Pankreas, Krabben Leber, Haifisch Darm, einem Schimmelpilz (Asp. oryzae Cohn) und Seesternen konnte die Saure gar nicht gespalten werden. Man kann vermuten, dass es Alginsaure spaltendes Enzym nur in den Tieren gibt, die auf alginsaurehaltigen Pflanzen, d. h. braunen Algen, fressen und leben.

Studies on the Vitamin D

III. Antirachitic Activation of Ergosterol by means of the Action of Ozone and on the Chemical Nature of Vitamin D.

By

Etsuo TAKAMIYA

(Received November 26th, 1930)

In the present investigation I attempted a feeding experiment to ascertain whether ergosterol becomes an antirachitically active substance after the action of ozone or not, in the same way as ergosterol becomes active after irradiation with ultra-violet rays. And the chemical nature of vitamin D was discussed with the experimental results obtained.

Ergosterol was isolated from beer-yeast by means of the Heidushka and Lindner's modification of which melting point was 154°C (uncorrected). The ergosterol in itself was antirachitically inactive on a feeding experiment. Shimazu's primitive ozone-generator with one-tube was especially employed in this experiment for the reason stated in the original paper. Ergosterol (20 mg.) was dissolved in 10 c.c. of olive oil and then ozonized for a definite time.

From the results of the feeding experiments it became experimentally certain that ergosterol becomes an antirachitically active substance after the action of ozone. And it became evident also that the rate of activation of ergosterol increased after the action of ozone and then reached a maximum potency and from this time further action caused a falling off in potency, the product becoming completely inactive (one can readily see the presence of such a phenomenon also in the case of the activation of ergosterol through the irradiation with ultra-violet rays). And it was suggested from my experimental results that J_1 mono-ozonide ergosterol will possibly be vitamin D. And also it was roughly concluded such that the imperfectly ozonized ergosterol is vitamin D.

Studies on the Specific Properties of Protease and Amylase from the Standpoint of the Adsorption Phenomena.

III. The Relative Observation between the Enzymic Power of purified Enzyme Solution prepared by the Enzyme Adsorption of Aluminium Hydroxide and the Nitrogen Content of the same Solution.

By

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(From the Research Laboratory of the Agricultural Technology, College of Agriculture, Hokkaido Imperial University)

(Received December 25th, 1930)

In our first paper, we had already reported on some specific properties between protease and amylase of Taka-diastase and pancreatin from the standpoint of the adsorption phenomena by using aluminium hydroxide and kaolin. Afterwards, in the second paper, we also described the results of the fractional separation of these two kinds of enzyme by the application of a selective adsorption of enzyme.

As we believe that it is one of the most important problems in the enzyme chemistry to separate the enzyme substance in a pure form as possible, we have further continued our experiment in which we had repeated the adsorption and then the elution of the enzyme from Taka-diastase, successively in five times by using aluminium hydroxide gel. The nitrogen content of each eluded solution and the enzymic power of the same solution were determined to know the relation between the enzymic power of purified enzyme solution and the amount of nitrogen contained in the same solution.

The results obtained from our experiments are shown in the following table.

Kind of enzyme solution	N-content (Ratio)	Enzymic power					
		Amylase				Protease	
		Sacch power	Lique power	Saccharase	Maltase	Gelatine decomp. power	Peptone decomp. power
Original solution	100.00	57.6	52.7	62.8	33.3	20.9	5.1
I-Elution	33.7	143.8	188.7	146.0	75.3	38.1	11.2
II- 〃	25.0	153.0	170.8	157.9	66.4	38.3	12 9
III- 〃	19.6	158.4	200.1	149 5	61.9	36.7	14.4
IV- 〃	15.6	160.1	218.0	146 9	51.0	38.9	13.8
V- 〃	12.2	173.0	232.9	155.8	45.5	39.9	12.1

Here, we have offered a new formula showing the enzymic power as follows; for instance, in a monomolecular reaction.

$$K=\frac{1}{t}\ \text{in}\ \frac{a}{a-x}$$

$$\text{Enzymic power}=\frac{\text{mg. Substrate}\times K}{\text{mg. N in Enzyme solution}}$$

instead of

$$\text{Enzymic power}=\frac{\text{mg. Substrate}\times K}{\text{mg. Enzyme Substance}}\quad\text{(a known equation).}$$

By using the former equation, a new proposed one, we can more easily determine the enzymic power than by using the latter, and it will be an important problem in the enzyme studies to use the pure enzyme solution made free from impurity. In this meaning, it is evident that our new formula for the calculation of enzymic power is more suitable by considering from the latest knowledge of the enzyme chemistry.

By the results of this experiment, we have proved that the enzymic power of the eluded solution seems to be nearly parallel to the nitrogen content of the same solution.

IV. The Separation of the Enzymes to be Adsorbable and Non-adsorbable by Kaolin, using the Adsorption Method, with special reference to the Nitrogen Content of Enzyme Solution.

We had observed that there are two kinds of enzyme fraction, i. e., to be adsorbable and non-adsorbable by kaolin, in a Taka-diastase enzyme solution. But all these enzymes were easily adsorbed by aluminium hydroxide. And we also observed that the nitrogen in an enzyme substance will be present accompanied with the enzymic power to some degree,

The conclusion of our experiment are summarized as follows:

1) The nature of the enzyme components in Taka-diastase observed by the kaolin adsorption is shown in the following table.

Kind of enzyme	Substrate	Adsorbent	
		Aluminium hydroxide	Kaolin
Amylase (Saccharification)	Sol Starch	+	—
Amylase (Liquefaction)	Starch	+	—
Dextrinase	Dextrin	+	—
Inulase	Inulin	+	±
Maltase	Maltose	+	±
Invertase	Saccharose	+	+
Katalase	Hydrogen peroxide	+	±
Lipase	Tributyrine	+	+
Protease	Gelatine	+	+
Protease	Peptone	+	+

+ adsorbed, — not adsorbed, ± partly adsorbed

It is very interesting matter that amylase is not adsorbed by kaolin, but invertase is well adsorbed and maltase is partly adsorbed by the same adsorbent.

2) From the enzyme solution prepared by the kaolin adsorption, it is able to be again purified by using the adsorption method with aluminium hydroxide. By the determination of the enzymic power of purified enzyme solution, the increase of enzymic power of the same solution to that of the original solution is 8～12 times in amylase, 8 times in dextrinase, 24 times in invertase and 14～17 times in protease respectively.

3) Nitrogen compound conteined in an enzyme solution, which was eluded by phosphate buffer solution after kaolin adsorption, was remarkably decreased its adsorptive offinity to aluminium hydroxide.

4) Through our experiment we made a new contribution in the enzyme chemistry by separating amylase component free from other enzymes from Taka-diastase, a preparation of the enzyme of Aspergillus oryzae.

Biochemical Studies of Bone. (First Report)

By

Hiromiti HARA.

(Received January 30th, 1931)

The author(1) has reported the "Sexual differences in the composition of bone" as the preliminary report of "Biochemical studies on bone, skin, hide and sheal".

The conclusions showed that the contents of both total nitrogen and phosphorus were higher in male, but calcium was higher in female. And when these datas were calculated to N=100, the contents of both calcium and phosphorus were higher in female than in male.

The author expected that the composition of bone must differ by its position. So that he divided the bone into the next four parts and examined.

Fore leg (scapula, radia, ulna, humerus): right and left.

Hind leg (femur, tibia, fibula): right and left.

The contents of the total nitrogen, ash, calcium and phosphorus in these samples were determined. Total nitrogen was determined by the Kjeldahl method, the contents of ash by ordinary method, calcium by Kramer–Tisdall's method(2) and phosphorus by Bell–Doisy–Briggs' method(3).

Determination of ash.

The results showed that the contents of ash are higher in the female than in the male, and the highest contents of ash are 79.92%, the lowest 59.95%.

Determination of phosphorus and calcium.

The datas showed that the contents of calcium in the body are not constant, but the contents of phosphorus of the body are higher in the right than in the left with the same leg and generally higher in the fore legs.

When the average of phosphorus in the four parts of the body are compared by sex, phosphorus are higher in female, while that of calcium showed no difinite tendencies.

Gassmann(4) offered the structure formulae of calcium–phosphorus combination in the bone as lollows:

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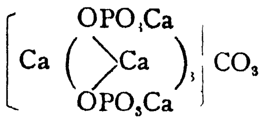
(1) H. Hara: Nippon Nogei Kwagaku Kwaisi, **6**, 655, (1930).

(2) B. Kramer and J. Tisdall: J. Biol. Chem, **47**, (1921).

(3) A. P. Briggs: J. Biol. Chem., **53**, (1922).

(4) Th. Gassmann: Z. physiol. Chem, **55**, 455, (1908).; Ibid. **70**, 161, (1910) Ibid. **83**, 403, (1913).



$$\left[\mathrm{Ca}\left(\mathrm{Ca}\begin{matrix}\mathrm{OPO_3Ca}\\ \mathrm{OPO_3Ca}\end{matrix}\right)_3\right]\mathrm{CO_3}$$

But the contents of calcium seemed to be inconstant. The author therefore expected, these variations must be caused by the different combination of bone-protein. And the author prepared collagen from bone and examined first its amino acid contents.

Preparation of collagen

The bones were removed from the body immediately after death. The adherent soft tissues were dissected and steeped in 0.1～0.2% HCl about 2～3 days. And then washed with distilled water and steeped into 0.1～0.2% NaOH and washed with distilled water, then dried with alcohol and ether. The contents of amino acid of these samples were determined by Van-Slyk's method.

The results showed that the contents of arginine are higher in male than in female.

On the Constitution of Tea Tannin.(1)

By

Michiyo TSUJIMURA.

(Received March 9 th, 1931.)

In the previous report, it was stated that tea-tannin, isolated by the author from green tea, is most probably the gallic acid ester of tea catechin(2) represented by the following formula:

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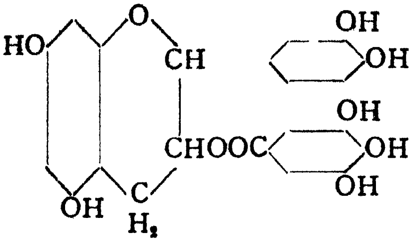
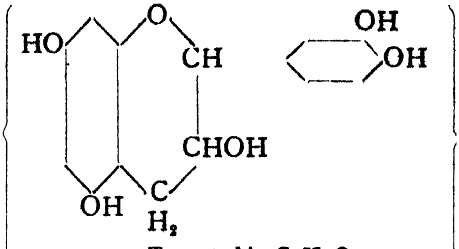
(1) M. Tsujimura: Bull Agricult Chem Soc. Japan, 6, 62 (1930).
(2) 〃 : 〃 〃 6, 70 (1930)



Tea tannin $C_{22}H_{18}O_{10}$



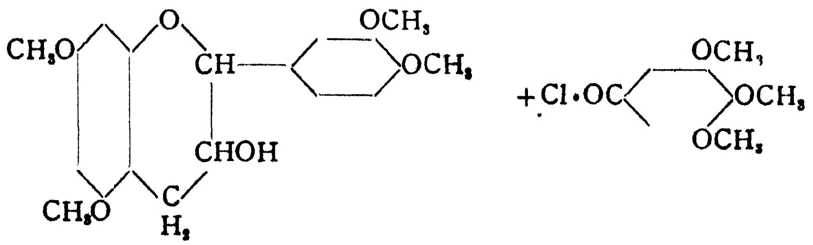
Tea catechin $C_{15}H_{14}O_6$

On acetylation, tea tannin forms heptaacetyl derivative corresponding to the formula, $C_{22}H_{11}O_{10}(COCH_3)_7$, and by boiling with 5% sulphuric acid it gives gallic acid and a reddish brown substance which yields phloroglucinol when heated with 50% potash. Unfortunately, tea catechin could not be detected among the hydrolytic products, because the latter easilly changes to a reddish brown substance when heated with sulphuric acid.

Continuing the studies on this subject, the author has now prepared a methyl derivative and proved it to be heptamethyl tea tannin $C_{22}H_{11}O_3(OCH_3)_7$

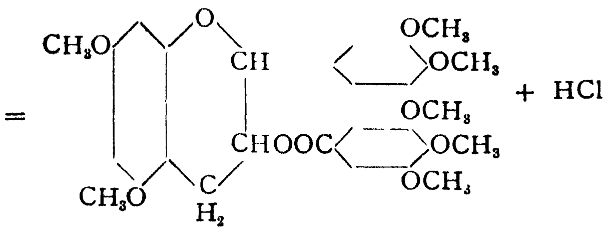
By oxidative decomposition with potassium permanganate, this compound gives trimethyl gallic acid $(OCH_3)_3C_6H_2CO_2H$ and veratric acid $(OCH_3)_2C_6H_3CO_2H$.

Although these facts are in accordance with the above assumption, yet more decisive proof must be forwarded by means of the direct synthesis of this compound, and so the author attained this by preparing heptamethyl tea tannin synthetically. For this purpose, tetramethyl tea catechin was coupled with the chloride of trimethyl gallic acid in presence of quinoline, according to the following scheme:



Tetramethyl tea catechin

Trimethyl gallyl chloride



Tetramethyl tea catechin–trimethyl galloate (Heptamethyl tea tannin)

The reaction proceeded smoothly and tetramethyl tea catechin trimethyl galloate $C_{22}H_{11}O_3(OCH_3)_7$, was obtained as colourless prisms or plates, melting at 140°. It shows no optical activity. The absorption spectrum of this compound (Fig. 3) was found to be identical with that of heptamethyl tea tannin (Fig. 4) prepared from natural tea tannin, while that of tetramethyl tea catechin (Fig. 1) or of trimethyl gallic acid (Fig. 2) was quite different from that of the latter (Fig. 4). The synthetical product when demethylated, forms an amorphous white powder which gradually oxidizes in the air to a reddish mass just like the natural tea tannin. Thus the author believes that the constitutional formula of tea tannin above stated is fully confirmed.

Experimental.

1. *Methylation of Tea Tannin.*

1.4 grams freshly prepared tea tannin were dissolved in 6 c.c. methyl alcohol, to which 5 c.c. dimethyl sulphate were added and treated with 5 c.c. 50% KOH drop by drop with shaking. The reaction product was poured into water, and after standing for an hour it was collected, washed with water and dried on a porous tile; the yield was ca. 1.4 grams. For purification it was dissolved in aceton, treated with a little animal charcoal, filtered and evaporated; the residue thus obtained was again dissolved in hot methyl alcohol, and the substance separated on cooling were dried first on a porous tile and afterwards in a desiccator containing conc. sulphuric acid.

The analysis gave following result.

	Sample	CO_2	H_2O	C%	H%
I.	3.178 mg.	7.335 mg	1.769 mg	62·95	6 19
II.	2.463 〃	5.722 〃	1.330 〃	63 36	6.00
	average			63.16	6.10
	calc. for $C_{22}H_{11}O_3(OCH_3)_7+\frac{1}{2}H_2O$			63.40	6 06

Thus the analysis proved it to be heptamethyl tea tannin.

Specific rotation of this compound in aceton solution: $[\alpha]_D^{19} = -135°$.

2. *Oxidation of Heptamethyl Tea Tannin with Potassium Permanganate.*

The oxidation was carried out according to the method of Perkin(3) as follows:

(1) Heptamethyl tea tannin was dissolved in methyl alcohol, heated on a water bath and a strong solution of potassium permanganate was added drop by drop until the red colour remained unchanged on standing. The excess of permanganate was then decomposed with sodium sulphite and filtered; the filtrate was neutralized with dilute sulphuric acid and extracted with ether. The etherial extract was evaporated and the crystalline residue thus obtained was treated with sodium bicarbonate to remove the phenolic substance and recystallized from hot water. Trimethyl gallic acid was thus obtained as colourless needles melting at 167°.

The analysis gave the following result:

Sample	CO_2	H_2O	C%	H%
2.776 mg.	5.743 mg.	1.483 mg.	56.43	5.93
calc. for trimethyl gallic acid $(CH_3O)_3C_6H_2CO_2H$			56.58	5.70

(2) 1.7 grams heptamethyl tea tannin were mixed with water and oxidized with permanganate in the same way as above-mentioned; the operation lasted for 30 hours. The crystals obtained thereby consisted of trimethyl gallic acid and veratric acid. The latter acid isolated from the mixture melted at 175°. Mixed with pure preparation of veratric acid, no depression of melting point was observed.

3. *Isolation of Tea Catechin.*

For the isolation of tea catechin, the method(2) previously mentioned was somewhat modified as follows:

The hot water extract of green tea (inferior quality) was treated with so much neutral lead acetate until no more precipitate was formed. The thick precipitate thus produced was filtered off, and the clear filtrate was treated with dilute caustic soda until faintly alkaline reaction was noticed. The yellow precipitate formed thereby was collected, washed with water and decomposed with hydrogen sulphide. The filtrate from lead sulphide was evaporated in vacuum to a small volume and repeatedly shaken with ethyl acetate. The united ethyl acetate extract was evaporated and the residue there-from was dissolved in hot water and filtered while hot. From the filtrate tea catechin crystallized out on cooling, which after recrystallization

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(3) A. G. Perkin: Soc., 87 398, (1905).

Tetramethyl tea catechin $\frac{1}{1000}$ mol.

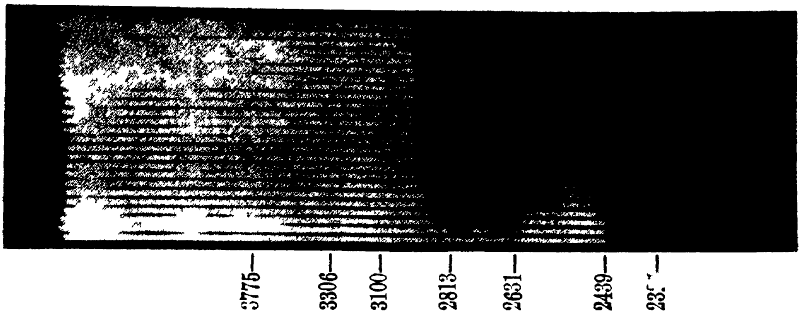

Fig. 1

Trimethyl gallic acid $\frac{1}{5000}$ mol

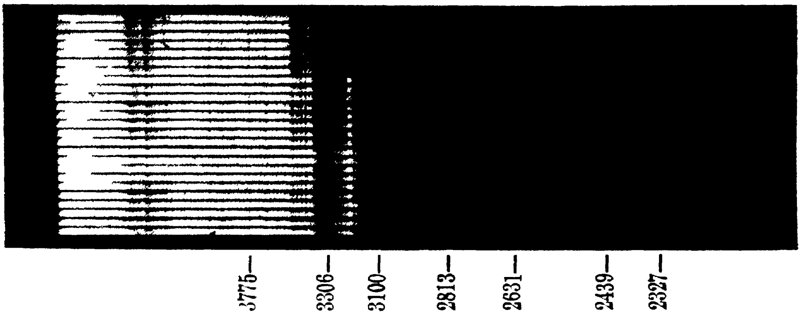

Fig. 2

Tetramethyl tea catechin-trimethyl galloate $\frac{1}{20000}$ mol.

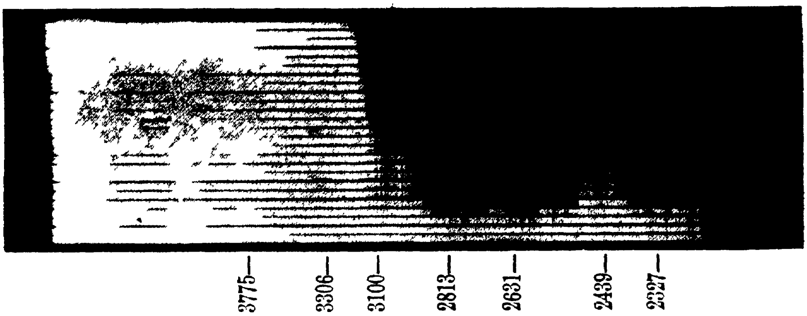

Fig. 3

Heptamethyl tea tannin $\frac{1}{10000}$ mol

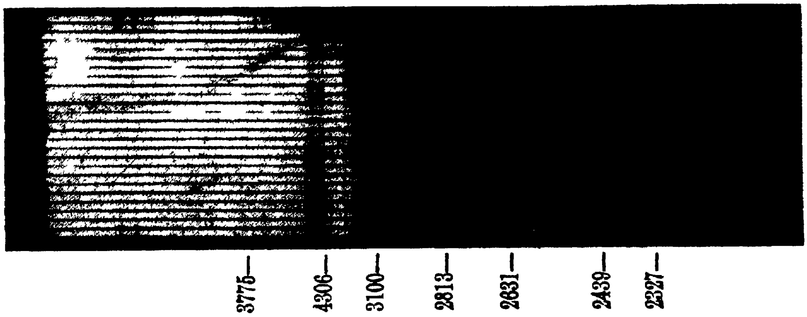

Fig. 4

M Tsujimura · On the Constitution of Tea Tannin.

melted at 237∼238°. The yield was ca. 0.11% of the green tea used.

4. *Tetramethyl tea catechin.*

The methylation of tea catechin was carried out as previously mentioned.(4) Tetramethyl tea catechin thus obtained melted at 153∼154°.

5. *Trimethyl gallic acid.*

The methylation of gallic acid was also carried out in the same manner as in the case of tea catechin. The methylated product was poured into water and warmed on a water bath at 60° for three hours. After cooling it was acidified with sulphuric acid, whereby the crystals of trimethyl gallic acid separated out, which was washed with water and dried on a porous tile; m. p. 167°. Mixed with trimethyl gallic acid obtained by the hydrolysis of heptamethyl tea tannin, it gave no depression of melting point.

6. *Trimethyl Gallyl Chloride.*

2 grams thionyl chloride were mixed with 0.5 gram trimethyl gallic acid in a glass tube, and warmed in a water bath. When the reaction was ended, the excess of thionyl chloride was removed by distillation in vacuum and the tube was immediately sealed.

7. *The Synthesis of Tetramethyl Tea Catechin–Trimethyl Galloate.*

The synthesis was carried out according to the method of E. Fischer.(5) For this purpose 0.4 gram tetramethyl tea catechin and 0.5 gram trimethyl gallyl chloride were dissolved in 2 c.c. of chloroform. 1 cubic cm. quinoline was added to it and the mixture was shaken for an hour. After standing for three hours, it was diluted with chloroform, and washed with dilute sulphuric acid and water successively. The chloroform solution was then evaporated under reduced pressure, the residue therefrom was dissolved in hot methyl alcohol and filtered while hot. After standing for some time, the crystals separated out, which after recrystallization formed colourless prisms or plates, melting at 140°.

The analysis gave following result:

	Sample	CO_2	H_2O	C%	H%
I	3.296 mg.	7.793 mg	1.825 mg.	64.48	6.15
II.	2.299 〃	5.465 〃	1.245 〃	64.98	6.16
			average	64.73	6.16
calc for tetramethyl tea catechin-trimethyl galloate $C_{22}H_{11}O_4(OCH_3)_7$				64.41	5.97

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(4) loc. cit.

(5) E Fischer und M Bergmann: B, 15, 1760, (1918).

The crystals dissolved in aceton showed no optical rotation.

The author expresses her sincere thanks to Prof U. Suzuki for his kind direction throughout this work.

On the Isolation of Phytosterolin from Wheat Embryo.

By

Nobuzo NAKAMURA and Akiyoshi ICHIBA.

(Received March 9th, 1931)

In the course of studying on the ether extract of wheat embryo,* we came to isolate a glucosidic substance, which we believed to be phytosterolin as designated first by F. B. Power and A. H. Salway and certified by them of natural occurrence.(1)

For the preparation, air dried wheat embryo powder was extracted exhaustively with ether. The oil obtained was perfectly clear at first, but after several days gave a small quantity separating out. It was collected and washed with hot ether and ethyl alcohol. This insoluble material was dissolved in a small amount of hot amyle alcohol. On standing, microscopic needle crystals were obtained; m. p. 285°~290°C (uncorr), yield 0.2%.

Acetate: m. p. 168°C (uncorr.), $[\alpha]_D = -22.4^\circ$ (in chloroform).
Benzoate: m. p. 198°C (uncorr.), $[\alpha]_D = +17.24^\circ$ (in chloroform).

On hydrolyzing, phytosterol with melting point 137°C (uncorr.) and glucose were obtained. The details were as follows:

Experimental.

In order to ascertain a pure material, the crude material thus obtained was acetylated with acetic anhydride, which was then recrystallized from ethyl alcohol in colourless, glistening leaflets, melting at 168°C (uncorr.).

The acetate was converted again into original state by saponifying and washing with water, alcohol, and ether.

Liebermann–Burchard's and Hager–Salkowski's reactions for sterols, as well as α–Naphtol reaction for carbohydrates were positive.

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* Not extra pure, but contaminated with bran.

(1) J. Chem Soc, 103 (1913), 3[illegible], 1022.

After being dried to constant weight at 100°C, it was analyzed.

Substance taken	0.0517 g.	CO_2	0.1359 g.	H_2O	0.0466 g.
	0.0536 ″		0.1424 ″		0.0486 ″
C%	71.71, 72.45			H%	10.01, 10.08
Average %		C%	72.08	H%	10.05
$C_{33}H_{56}O_6$ requires		C%	72.30	H%	10.20

Acetyl derivative.

A portion of this material was converted into an acetate by the ordinary method of heating for 30 minutes with an excess of acetic anhydride on the sand bath. The product was recrystallized from ethyl alcohol in leaflets, melting at 167∼168°C (uncorr.); $[\alpha]_D = -22.4°$ (0.6236 g. in 25 c.c. $CHCl_3$).

After being dried to constant weight at 100°C, it was analyzed.

Substance taken	0.0417 g.	CO_2	0.1048 g	H_2O	0.0328 g.
	0.0476 ″		0.1201 ″		0.0380 ″
	C%	68.59,		H%	8.71
		68.80			8.81
	Average %	68.70			8.76
$C_{33}H_{52}O_6(COCH_3)_4$ requires	C%	68.67,		H%	8.94

Determination of molecular weight.

Substance taken 19.77 mg. camphor taken 101.28 mg.
$\Delta = 10.75$, mol. wt. $= 726.7$. Calc. 716.

Determination of acetyl value by Freudenberg's method.

Substance taken	0.3020 g.	8.29 c.c.	1/5 N	KOH.
	0.2687 ″	8.73 ″	1/5 N	KOH.
Therefore	CH_3CO %	24.44	23.43	
	Average %	23.93,	Calc.	24.01.

Benzoyl-derivative.

Heating with an excess of benzoic acid anhydrous for 30 minutes on sand bath, it was poured into alcohol and neutralized with potassium carbonate, then after adding water, extracted with ether repeatedly. On evaporating the ether, the residue was recrystallized from a mixture of alcohol and ether in slender colourless needles, melting at 198° (uncorr.); $[\alpha]_D = +17.24$ (0.1044 g. in 10 c.c. chloroform).

After being dried to constant weight at 100°C, it was analyzed.

Substance taken	0.0438 g.	CO_2	0.1224 g.	H_2O	0.0295 g.
		C%	76.20	H%	7.49
$C_{33}H_{52}O_6(COC_6H_5)_4$ requires		C%	75.90,	H%	7.52

Hydrolysis of phytosterolin.

It was ressolved in 60 c.c. of hot amyl alcohol and 20 c.c. of 15% HCl added, together with sufficient alcohol to form a homogeneous liquid. After heating for three hours under a reflux condenser, steam was passed through the mixture to remove the amyl alcohol, then solid substances were separated out.

The content of the flask was filtered and a solid substance was collected. This material was recrystallized several times from hot alcohol in glistening leaflets, melting at 137°C (uncorr.); $[\alpha]_D = -42°$ (62 mg. in 10 c.c. chloroform).

Liebermann–Burchard's and Hager–Salkowski's reactions were positive.

After being dried to constant weight at 100°C, it was analyzed.

Substance taken 0.0544 g.	CO_2	0.1672 g.	H_2O	0.0580
	C%	83.82	H%	11.84
$C_{27}H_{46}O$ requires		83 93		11 91

A portion of this substance was acetylated and another benzoylated, which were melted at 127°C and 143.5°C respectively. It showed no depression of melting point by mixtured test with phytosterol from other sources.

After being dried to constant weight at 100°C, they were analyzed.

Acetate taken 3.182 mg.	CO_2	9.526 mg.	H_2O	3.249 mg.
	C%	81.64	H%	11.34
$C_{27}H_{45}O(COCH_3)$ requires	C%	81.24,	H%	11.29,
Benzoate taken 1.925 mg.	CO_2	5.838 mg.	H_2O	1.818 mg.
	C%	82.71	H%	10.49
$C_{27}H_{45}O(COC_6H_5)$ requires	C%	83.19,	H%	10.28.

Determination of sterol content.

0.01 gram. of acetyl derivative was dissolved in 60 c.c. of alcoholic hydrochloric acid solution (1.5%) and heated for six hours under a reflux condenser. After the hydrolysis was completed, it was extracted with ether and on evaporating the ether, the residue was dissolved in 95% alcohol and precipitated with 1% digitonin solution in 90% alcohol.

The weight of precipitate was 0.0230 g.

As sterol 0.00534 g.	therefore sterol %	53.42
$C_{33}H_{52}O_6(COCH_3)_4$ requires		53.92

Determination of glucose.

The aqueous solution, from which sterol had been separated by extraction with ether, reduced the Fehling's solution, showing the presence of sugar. The Bial's and Tollen's reactions were negative and the Seliwanoff's reaction was positive. It formed osazone with phenylhydrazine. From these tests it was thought to be glucose.

Rotation of the aqueous solution.

0.4598 g. of the original substance was hydrolyzed by the above described method and the aqueous solution from which phytosterol was separated off, was neutralized with sodium carbonate and concentrated to 25 c.c.

The rotation of this solution was observed.

$l=2$ $\alpha=0.64$ $[\alpha]_D=52.9°$

The specific rotation of glucose is 52.6°.

Preparation of glucosazone.

1.2 grams of this substance were hydrolyzed and the aqueous solution obtained was exactly neutralized with sodium carbonate, evaporated to dryness, the residue was digested with absolute alcohol and filtered.

On evaporating the alcoholic filtrate, a small amount of syrupy residue was obtained, which was dissolved in water together with 0.75 g. phenylhydrazine hydrochloride and one gram of sodium acetate. It yielded one gram of osazone.

It was collected and washed with methyl alcohol and recrystalized from ethyl alcohol in yellow typical needle crystals, melting at 207°C (uncorr.). It melted at 207°C by mixing with synthetic glucosazone.

After being dried at 100°C, it was analyzed.

Substance taken 3.028 mg. N=0.390 c.c. $p=767.95$ $t=20°C$

N% 15.2 Calc. N% 15.6

Summary.

We have isolated the phytosterolglucoside from wheat embryo powder and proved that it was identical with the phytosteroline which had been prepared from other sources and so designated by Power and Salway.

The authors wish to express their sincere thanks to Prof. U. Suzuki for his advices and encouragements throughout this work, and also thanks to Mr. T. Nanbo for micro-analysis.

On the Nutritive Value of Pentosan. I.

The Effect of Xylan on the Fat Formation.

By

Hisayoshi IWATA

(Morioka Imperial College of Agriculture and Forestry)

(Received May 5, 1930)

Introduction.

During his notable researches on the productive value of crude fiber and other nutrients, Kellner once conjectured that the fat forming value of straw pentosan would be equivalent to that of starch. No direct evidence or strict confirmation of this assumption has, however, ever been presented inspite of the abundant distribution of pentosans in feeding stuffs and food materials, especially in cereal straws.

So, taking xylan as one of the examples of pentosans, I have investigated its productive value in comparison with that of starch.

Experimental.

Xylan was prepared mainly from the alkaline extract of rice straw by the addition of alcohol. This xylan contained 85.7% of organic matter, 86.2% of which was xylan according to the yield of furfurol phloroglucid or of non-fermentable sugar. Its total fuel value was 4167 calories per gram of organic matter.

Starch that was used as a comparison contained 80.6% of organic matter with fuel value of 4136 calories per gram.

Metabolism experiments were carried on for about 100 days, dividing them into 3 or 4 periods of a definite diet such as basal ration, basal + xylan or basal + starch. As the basal ration 160 grams of the mixture, which consisted of 50% of "Genge" hay, 45% of barley powder and 5% of dried radish leaf powder, were used. The metabolism experiments on carbon and nitrogen, made afterwards, proved that this basal ration was adequate and slightly in excess of the maintenance requirement for both of the experimented animals. In the period of "basal + xylan" or "basal + starch" 20 grams of the air dried sample were daily added to the basal ration. On account of the occurence of some amounts of digestibility depression, the digested amount of xylan was directly calculated from the digestibility coefficient of pentosan and the starch from that of the total inverted sugar.

The income and output of carbon and nitrogen were estimated from the analysed results of fodder, feces, urine and expired gas and also the nutritive ratio of each diet was calculated. Respiration experiments were made twice a period applying Pettenkofer's respiration apparatus installed in professor Katayama's laboratory at the Kyushu Imperial University. It must be added here that the formation of methan gas by the rabbits were negligible.

The results of the metabolism experiments mav be summarised in the accompanying table (Unit in grams):-

Rabbit		Rabbit No. I.							
Period		Basal		Basal + Starch		Basal + Xylan		Basal	
Carbon or Nitrogen		C	N	C	N	C	N	C	N
Income	Basal ration	64.51	3.176	64.51	3.176	64 51	3.176	64.51	3.176
	Sample	0	0	7 16	0.004	7.85	0.019	0	0
Out put	Feces	29.22	1.378	31.77	1 554	33.50	1.494	28.69	1.332
	Urine	2.26	1.600	2 20	1 381	2.13	1.391	2.28	1.683
	Expiration	31.80	0	34.76	0	33.86	0	32.63	0
Gain	Stored inbody	1.23	0.198	2 94	0.245	2.87	0.310	0.71	0.161
Protein, stored		1.172		1 450		1.834		0.953	
Fat, stored		0.80		2.84		2 48		0.27	
Stored, sum as fat		1.49		3.70		3.56		0.83	
Nutritive ratio		6.04		7.79		7.21		5 90	

Rabbit		Rabbit No II					
Period		Basal + Starch		Basal		Basal + Xylan	
Carbon or Nitrogen		C	N	C	N	C	N
Income	Basal ration	64.51	3.176	64 51	3 176	64.51	3.176
	Sample	7.16	0.004	0	0	7.85	0.019
Out put	Feces	34.01	1.674	31.24	1.608	34.57	1.638
	Urine	2.10	1.298	1 81	1.347	2.13	1.291
	Expiration	31.62	0	31.10	0	31.40	0
Gain	Stored in body	3.94	0.208	0.36	0 221	4.26	0.266
Protein, stored		1.231		1.308		1.574	
Fat, stored		4.30		−0.43		4.48	
Stored, sum as fat		5.03		0.34		5.41	
Nutritive ratio		7.99		6.51		7.66	

The amount of fat accumulated in the body from one kilogram of digestible xylan or starch and also the percentage of production calorific valu per calorific value of the digested nutrients were computed :-

Nutrients	Rabbit No	Fat accumulated from 1 kg digested nutrients	Production caloric
Xylan	1 2	197.2 g 341.0 } average 269.1 g	61.3%
Starch	1 2	202.7 325.0 } average 263.9 g	60.5%

The excreted urine of each period was analysed as to its total sugar as well as pentose contents, the amount of the latter was corrected for the presence of gluculonates and other urine constituents according to my equation,

$$(\text{Urine, Phloroglucid-glucuronate, Phloroglucid} \times 0.422) \div 0.692 \times 1.0784 = \text{Urine pentose.}$$

The results obtained may be tabulated as follows :-

Period	Rabbit No. 1				Rabbit No. 2		
	Basal	Basal + Xylan	Basal + Starch	Basal	Basal + Starch	Basal	Basal + Xylan
Total sugar per day mg	455	481	510	446	446	403	500
Pentose 〃 〃	73	73	109	66	78	83	81

Conclusion

Nearly all of the digested xylan was metabolised by the rabbits so that mere trace of organic matter such as pentose etc. was excreted in the urine.

The productive value of digested xylan was equal to that of starch as Kellner had assumed. In other words, the amount of fat formation, calculated from the metabolism experiments on carbon and nitrogen, was 269 grams from one kilogram of digestible xylan and the productive calorific value, which was calculated from the accumulated fat, was 3029 calories per gram of digestible xylan, or the value corresponding to 61% of the calorific value of digested xylan.

On the Nutritive Value of Pentosan. II.

The Effect of Xylan Administration on the Glycogen Formation and Blood Composition.

By

Hisayoshi IWATA.

(Received May 7, 1931)

In the first report of this investigation it was concluded that, the amount of fat formed from the digested xylan is equal to that formed from starch.

According to this conclusion the amount of glycogen formed from xylan must also be equal to that formed from starch, but this point has not been experimentally investigated. Slowtzoff has once reported that, some amounts of pentosan could be found in the rabbit's body after the administration of xylan. The purpose of the present investigation was to ascertain the formation as well as the amount of glycogen or pentosan in the rabbits, administered xylan. The effect of xylan on the blood composition was also studied in this connection.

Methods

Male rabbits of medium weight were kept fasting in metabolic cages. Each animal was armed with a tin belly-band in order to prevent its access to feces. Room temperature was kept between 10° and 15° C. After a period of fasting ranging from 32 to 40 hours, either xylan or starch was administered into the stomach by the uretheral catether, in amount of 5 g. per 1 kg. of body weight. The blood samples were obtained from marginal ear veins at intervals using potassium oxalate as an anticoagulant. The blood sugar was analysed by the method of Hagedorn and Jensen, the reducing power after fermentation by an ordinary method, haemoglobin by the Sahli's haemometer method, and non-protein nitrogen by the Bang's micro Kjeldahl method. About 50 hours after fasting started, each rabbit was killed and its glycogen contents of liver as well as muscle were determined by the Pflüger's method. Glucose resulting from the hydrolysis of the glycogen was determined by the Bertrand's method. In order to ascertain the presence or absence of pentosan in the liver, the pentose content in the hydrolysed solution of glycogen, which was precipitated by the Pflüger's method as above noted, was determined both by the Tollen's method and by the fermentation method. The amounts of xylan, starch or sugar contained in the digestive tracts were determined by the usual method. The urine sugar was determined from the urine excreted as well as that remained in the bladder. The controll rabbits were kept fasting 48 hours and passed through same examination as above mentioned.

Results

The results obtained may be shown by the following tables.

The amounts of glycogen stored in each rabbit were calculated as follows:

(Liver weight × Liver glycogen %) + (Body weight × 0.47 × Muscle glycogen %)

The amounts of glycogen formed either from xylan or starch were calculated by the subtraction of the amounts of glycogen found in the controll rabbits (fasted) from that of the animal administered. The results may be represented by the following graph.

Table I. Effect of Xylan.

Rabbit No.	Date. 1930 1931	Time (af. = after) (° = hrs)	Treatment and remark	Blood sugar mg. in 100c.c	Nonfermentable blood sugar mg. in 100c.c.	Nonprotein nitrogen mg. in 100c c.	Haemoglobin g. in 100c c	Liver weight g.	Liver glycogen mg. in 100 g	Muscle glycogen mg. in 100 g.
1	Oct 21	am. 10.0	Fasting started							
	23	"	Body weight 1 8 kg 10 g Xylan given							
		af moment	Blood taken	105	39	48	17.0			
		af. 4°	"	93	37	52	17 1			
		af '	Killed 52° after fasting Body weight 1.68kg					56	78	39
2	Nov.11	am. 10 30	Fasting started							
	12	" 11.30	Blood taken.	108	43	51	16 4			
		p m. 4 30	Body weight 1.9 kg 10 g Xylan give							
	13	af. 17° 10′	Blood taken	115	46	46	17 0			
		af. 18° 0	Killed 48° after fasting Body weight 1 75kg Urine sugar 110 mg. for 18° 1 g Xylan recovered in stomach					59	1139	174
3	Dec.25	p.m. 3.00	Fasting started							
	27	a.m. 7 55	Body weight 1.44 kg 8 g Xylan given							
		af 25′	Blood taken	92	41	49	15 9			
		af. 4°	"	—	43	44	16 1			
		af.' 8°05	"	85	38	54	16.0			
		af. 9°15	Killed 50°10′ after fasting Body weight 1 3 kg. Urine sugar 173 mg for 9°. 1 g. Xylan recovered in stomach					40	890	97
4	Dec.26	a m. 8.35	Fasting started.							
	27	a m. 9.25	Blood taken.	102	32	53	15.9			
		p m. 5 20	"	108	36	—	—			
		p m. 5.30	Body weight 1.84 kg 8 g Xylan given.							
	28	af 15°50′	Blood taken.	117	35	56	15.9			
		af. 16°20′	Killed 49°15′ after fasting. Body weight 1.75 kg. Urine sugar 235 mg for 16°. 1.5 g. Xylan recovered in stomach					57	749	161

5	Jan. 9	a.m. 9.10	Fasting started.							
	10	p.m 5 30	Blood taken.	99	31	—	17.1			
		p m. 5.45	Body weight 1.8 kg. 9 g. Xylan given.							
		af. 15°15′	Blood taken.	112	32	47	16.9			
		af 15°30′	Killed 48°05′ after fasting. Body weight 1.65 kg 0.7 g Xylan recovered in stomach. and intestine.					52	1493	90
6	Feb. 5	p.m. 1.40	Fasting started.							
	7	a.m. 8.40	Body weight 1.9 kg 10 g. Xylan given.							
		af. 5′	Blood taken.	87	29	46	17.3			
		af. 7°50′	〃	98	31	43	17.1			
		af. 8°	Killed 51° after fasting. Body weiget 1,8kg. Urine sugar 86 mg for 8°. 1.85 g. Xylan recovered in caecum, 0.3 g. in stomach.					62	1020	220
			Average (No. 2～6)						1058	149

Table II. Effect of Starch.

Rabbit No.	Date. 1930 1931	Time (af. = after) (° = hrs)	Treatment and remark	Blood sugar mg in 100c c.	Nonfermentable blood sugar mg. in 100c.c.	Nonprotein nitrogen mg in 100c c.	Haemoglobin g in 100c.c.	Liver weight g.	Liver glycogen mg. in 100 g.	Muscle glycogen mg. in 100 g.
7	Oct. 3	p.m 0.10	Fasting started.							
	5	p.m. 0.30	Body weight 1.95 kg. 10 g. Starch given.							
		af moment	Blood taken	92	31	44	17.3			
		〃 2°50′	〃	98	36	49	17.3			
		〃 4°	Killed 52°20′ after fasting. Body weight 1.82 kg					60	405	92
8	Oct. 9	Pm. 0	Fasting started.							
	11	〃	Body weight 1.70 kg. 10 g. Starch given.							
		af. moment	Blood taken.	92	38	50	17.3			
		〃 2°20′	〃	93	—	—	17.3			
		〃 3°50′	〃	103	40	49	17.0			
		〃 4	Killed 52° after fasting Body weight 1.65kg.					46	274	75

9	Oct. 29	p.m. 0.30	Fasting started.							
	Nov. 1	〃	Body weight 1.5 K. 10 g. Starch given.							
		af. 25′	Blood taken.	79	—	—	15.4			
		〃 2°	〃	93	—	—	15.2			
		〃 4°05′	〃	87	—	47	15.6			
		〃 4°20′	Killed 52°20′ after fasting. Body weight 1.5 K.					50	326	84
10	Nov.18	a.m. 10.30	Fasting started.							
	19	〃 10.30	Blood taken.	96	42	41	17.1			
		〃 10.35	7g Starch given.							
		p.m. 4.30	Blood taken	94	—	39	17.0			
	20	a.m. 10.30	〃	103	40	44	17.3			
	20~26		Normal feeding.							
	Nov.26	a.m. 9.20	Fasting started again							
	27	p.m. 4.0	Body weight 1.35 kg. 6 5 g. Starch given.							
	28	af. 17°20′	Killed 48° after fasting. Body weight 1.32kg.				17.4	39	727	62
11	Dec. 9	a m 9.30	Fasting started							
	10	〃 10.00	Blood taken	108	41	39	17.0			
		p.m. 4.00	〃	90	—	37	16 4			
		〃 4.30	Body weight 1.65 kg. 8 g. Starch given							
	11	af. 17°35′	Blood taken	125	45	39	17.0			
		〃 18°	Killed 49° after fasting Body weight 1.64kg. Urine sugar 213 mg. for 18°. 2 g Starch recovered in stomach.					50	660	138
12	Dce.25	p.m. 4.00	Fasting started.							
	27	a m 8.40	Body weight 1.85 kg. 8 g. Starch given.							
		af. 20′	Blood taken	93	41	50	15.6			
		〃 3°55′	〃	88	—	—	16.0			
		〃 8°20′	〃	—	40	52	15.9			
		〃 9°	Killed 49°40′ after fasting. Body weight 1.38 kg Urine sugar 189 mg. 1.4 g. Starch recovered in stomach.					42	704	87
13	Jan. 6	p.m. 5.00	Fasting started.							
	8	a.m. 9.00	Blood taken	104	46	48	16.2			
		〃 9 15	Body weight 1.8 kg. 9 g. Starch given.							
		af. 7°15′	Blood taken	105	40	45	16.5			
		〃 8°	Killed 48°15′ after fasting. Body weight 1.81 kg, 0.7 g. Starch recovered in stomach.					62	1538	356
			Average (No. 10~13)						907	161

Table III. Effect of Fasting.

Rabbit No.	Date. 1930 1931	Time (° = hrs)	Treatment and remark	Blood sugar mg in 100c c.	Nonfermentable blood sugar mg. in 100c c.	Nonprotein nitrogen mg in 100c c	Haemoglobin g in 100c.c.	Liver weight g.	Liver glycogen mg in 100 g.	Muscle glycogen mg. in 100 g.
14	Oct. 5	a.m. 10.0	Fasting started.							
	6	p.m 2.0	Blood taken Body weight 1.84 kg	108	39	46	17.1			
	7	a.m. 10.0	Killed 48° after fasting Body weight 1.8kg.					50	109	90
15	Oct. 12	a.m. 10.00	Fasting started.							
	13	〃 9 30	Blood taken Body weight 1.70 kg.	86	43	48	16.9			
	14	〃 9 30	〃	78	40	52	17.5			
		〃 10.00	Killed 48° after fasting Body weight 1.6kg.					41	74	32
16	Nov. 8	p.m 3.0	Fasting started.							
	10	〃 3.0	Killed 48° after fasting. Body weight 1.3kg.					44	73	41
17	Jan. 10	a.m. 10.00	Fasting started							
	11	p.m. 5.00	Blood taken. Body weight 1.5 kg	93	38	48	16.7			
	12	a.m 9.50	〃	89	35	47	16.9			
		〃 10.00	Killed 48° after fasting Body weight 1.45kg. Urine sugar 243 mg. for 18°					45	133	39
18	Jan. 10	a.m 10.00	Fasting started.							
	11	p.m. 5.10	Blood taken Body weight 1.4 kg	87	41	44	—			
	12	a.m. 10.30	Blood taken.	86	44	43	16 9			
		〃 10.35	Killed 48°35′ after fasting. Body weight 1.38 kg. Urine sugar 196 mg. for 20°.					45	108	79
			Average (No 14~18)						99	56

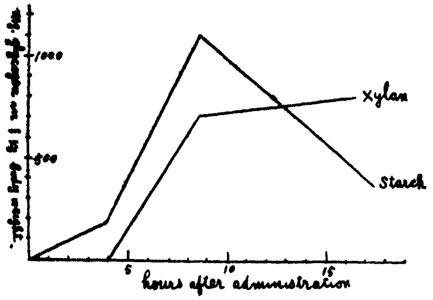

Fig. Glycogen increased in Liver and Muscle.

The presence or absence of pentosan in the liver of rabbits, to which xylan was administered, was investigated with the following results.

Table IV. Pentosan (?) found in Liver.

Administered	Rabbit No	Time	Pentosan (?) %	
			By Tollen's method	By fermentation method
Xylan	4	1930, Dec 26~28	0.019	—
	5	1931, Jan. 9~11	0.036	0.04
	6	〃 Feb. 5~7	0.011	—
Starch	10	1930, Nov 26~28	0.011	—
	12	〃 Dec. 25~27	0.021	—
	13	1931, Jan 6~8	0.088	0.05

Conclusion

1. The amount of fermentable blood sugar was found increased by 0.1 %, 8~10 hours after the administration of xylan, but the amounts of non-fermentable blood sugar, non-protein nitrogen and haemoglobin remained unchanged.

2. No pentosan was found in the rabbit's liver even when some amounts of xylan was administered.

3. The total amounts of glycogen formed by the administration of xylan were nearly equal to that formed when the same amount of starch was given.

4. The results above stated is a strict confirmation of the conclusion, stated in the first report i. e. the nutritive value (dynamic energy) of the digested xylan is equal to that of starch so far as the rabbit, nay, probably the herbivorous animals are concerned.

5. Digestion velocity of xylan is smaller than that of starch.

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Investigation on Cellulose Decomposition in Soils. I.

Re-examination of some stock cultures.

By

Arao ITANO and Satiyo ARAKAWA.

(Received May 2, 1931)

An enquiry was made as to the causal agent of forming the clear zone around the colony when the cellulose decomposing bacteria are cultivated on cellulose agar since there has been a dispute among various investigators, if the zone is formed by the enzymatic action or acid which is produced as the metabolic product.

The following cultures which were obtained from American Type Culture Collection, were used: Cellulomonas flavigena, fibula, fima, liquata, biazotea, cellasea, gelida, perurida and uda.

Various media were used such as McBeth, Lohnis and Lochhead, Skinner, Bradley and Rettger, Snieszko, Dubos and the author's media, a) feces and b) yeast extract.

The following summary are given:

1) Genus, Cellulomonas produce the enzymatic zones on cellulose agar medium; 2) the kind of media plays an important rôle and the organic nitrogen is the best source of nitrogen especially the digested casein and yeast extract; 3) all the cultures retained their activity after years storage although Cellulomonas uda, fima and gelida manifested strong power of the cellulose decomposition while Cellulomonas cellasea was the weakest.

Investigation on Electrometric Method for Determination of Chloride.

By

Arao ITANO.

(Received May 2, 1931)

The use and setting up of portable pH appratus (Itano) is described in conjunction with the Best's electrometric method for determination of chloride which uses a reference electrode of known potential.

The reference electrode is made up of a buffer solution exclusive of chloride, pH 3.00~3.03 to which quinhydrone is added. The silver metal

electrode coated with silver chloride by electrolysis is immersed in a sample and used as an indicator electrode. These two electrodes are connected to form a chain by means of an agar bridge which is made with saturated KCl and 3% agar. The chain is connected to the apparatus and the titration is carried out with the standard silver nitrate added from a burette. The end-point of titration is indicated by the reverse direction of galvanometer needle.

The special merits of the method are summarized as follows: 1) the end-point is sharp and easily observed which avoids the personal factor; 2) no necessity of making graph or any other interpolation like in case of other electrometric method; 3) each determination is carried out quickly, and no color and turbidity interferes with the method.

The portable pH apparatus (Itano) is very conveniently employed in completing the necessary equipment.

Determination of Chloride in Soils.

By

Arao ITANO and Akira MATSUURA.

(Received May 2, 1931)

The electrometric method described by the senior author is used in determining the chloride content in the soil samples which were collected along the irrigation canal running from the Takahashi river to the Kojima Bay in Okayama prefecture. Some samples were taken from the arable rice-field and the others, from the virgin field located on the bank or basin of the canal. The chromate method was applied at the same time to check each other.

The following summary and conclusions are given: 1) the results obtained by the electrometric method are in close agreement with those obtained by the standard chromate method; 2) there is a slight discrepancy among the results when the chloride content is very low; 3) the chloride content decreases gradually toward the source of irrigation in both arable and virgin soils; 4) the virgin soils contain much more chloride naturally than the arable soils of which the chloride has been gradually washed out; 5) the organic matter contained as much as in these samples does not interfere with the determination.

Chemical Studies of Agar-Agar.

I. The Rate of Hot-Water-Hydrolysis of Agar-agar.

By

Eiji TAKAHASHI and Kiyoshi SHIRAHAMA.

(Received May 8, 1931)

We are now studing the verious carbohydrates which separated from the "hydrato-kanten*" obtained by hot-water-hydrolysis of agar-agar. In this paper the rate of hydrolysis of various agar-agars and the general nature of the hydrolyzed solution are reported.

Samples used in these experiments were a purified bar kanten made in Japan (made mainly from *Gerdium Amansii*, Lamx.), Merk's agar-agar and that made in Karafuto (made from *Ahnfeltia plicata*).

The agar-agar and water were mixed in the ratio of 1 to 20 in weight and heated in autoclave at 130°C for certain hours of various intervals. Chemical and physico-chemical investigations were made upon the above resultant solutions and also the specific viscocity (I), hydrogen ion concentration (II), reduction value (III), aldose value (IV), free-sulfuric acid (V) and the quantity of humus (VI) were measured as follows :-

Table I.

The rate of hot-water-hydrolysis of Japanese bar Kanten

Time of heating (Hours)	I	II (PH)	For 100 g of Kanten			
			III*	IV	V	VI
2	2.514	4.61	5 g	12 g.	0.10 g	—
3	1.118	3.49	23	30	0.15	—
4	1.109	3.29	23	31	0.26	1.48 g
5	1.109	3.08	28	33	0.75	1.50
6	1.030	2.80	50	39	1.53	2.24
7	1.020	2.52	55	42	2.25	3.60
8	1.009	2.04	55	43	3.11	4.64

* Calculated as a galactose

Table II.

The rate of hot-water-hydrolysis of various agar-agar.

Time of heating (Hours)	Specific viscosity			Per cent for agar-agar					
				Reducing sugar			Free-sulfuric acid		
	B	M	K	B	M	K	B	M	K
2	2.51	—	—	5	—	—	0.10	—	—

---

* All hydrolyzed products of agar-agar.

3	1.12	1.83	4.04	23	12	5	0.15	0.22	0.03
4	1.11	1.25	1.70	23	19	13	0.26	0.37	0.07
5	1.11	1.20	1.40	28	30	28	0.75	0 59	0.08
6	1.03	1.15	1 18	50	44	28	1.53	0.67	0.13
7	1.02	1 07	1.12	55	51	29	2.25	0 85	0 18
8	1.01	1.00	1.09	55	51	42	3.11	0.96	0.24

B ·····Bar kanten　M ···· Merk's agar-agar　K······Karafuto kanten

When the above result is observed, tracing curves (ordinate······factors, abcissa······time of heating), it is found that the two minimum points of the viscosity coincide respectively with the two maximum points of the curves of the reduction value.

The marked discending of viscosity at the early stage may depend upon the disappearing of starchy substance which appears in the hydrolyzed solution heated for 2 hours in the case of the bar kanten. In the course of cooling of the resultant hydrolyzed solution, it exists in a white turbid state at first, but is precipitated soon after forming a starchy-like layer, the upper solution remaining clear.

It was also indicated that the aldose value is higher than the reduction value.

Protein of "Wakame" (*Undaria pinnatifida*).

I. Hydrolysis of "Wakame".

By

Shun'ichi Tase.

(Received May 12, 1931)

The material of Wakame was hydrolysed with hydrochloric acid. The proportion of various forms of amino nitrogen in the hydrolysed solution was determined by the Van Slyke's method as follows :-

The proportion of Amino Nitrogen in Wakame.

	Percentage in total N.	Percentage in the total soluble N, after hydrolysis
Soluble N	92.01	
Insoluble N (in the residue after hydrolysis)	8 75	
Ammonia N.	7.87	8 55
Melanin N	11.63	12.64
Precipitated with $Ca(OH)_2$.	3.51	3.81
Not precipitated with $Ca(OH)_2$, but with phosphotungstic acid.	8.12	8.83

Basic N.	**6.59**	**7.16**
Free amino N.	**3.92**	**4.27**
Non-amino N.	**2.67**	**2.89**
Arginine N.	**3.29**	**3.58**
Cystine N.	**2.93**	**3.18**
Nitrogen in the filtrate from base.	**61.69**	**67.04**
Free amino N.	**54.55**	**59.29**
Non-amino N.	**7.14**	**7.75**

From another one kilogram of the same sample various pure amino acid crystalls were separated by the ester method. Yeilds were as follows:

Yeilds of Amino Acids from 1 kg. of Wakame:

Amino Acid	Yeilds (g.)	Amino Acid	Yeilds (g.)
Glycocoll	0.80	Glutamic acid	8.90
Alanine	8.15	Proline	2.90
Valine	0.78	Anhydride of amino acid	0.08
Leucine	1.15	Phenylalanine hydrochloride (?)	5.36
Aspertic acid	1.58		

Studies on the Dietary Properties of Soybean Cake Flour Produced by the Alcohol Extraction Process.

I. Digestibility of Protein Supplied by Alcohol Extracted Soybean Cake.

By

Seiichi IZUME and Yoshinori YOSHIMARU.

(From the Central Laboratory, South Manchuria Railway Co.)

(Received May 11, 1931)

Introduction

Although soybean has been used to a considerable extent for food purposes in Japan and China, the soybean cakes, by-products of oil mills, have not yet been successfully introduced into the human dietary, a tremendous amount of the cakes produced in Manchuria being at present utilized almost exclusively as fertilizers and stock feeds.(1)

Some of the chief reasons why soybean cakes find such a limited use as human food may be pointed out as follows:

(1) The soybean cakes commonly produced in Manchurian oil mills, both press-cake and benzine extracted cake, possess a somewhat disagreeable odor and unpleasant taste and are of a yellow or brown color.

(2) They contain sand, dust, grasses and various other impurities as the oil is not expressed or extracted under sanitary conditions.

(3) The round press-cake easily becomes mouldy and rancid as a result of its high moisture content.

Dr. Sato and his co-workers(1) of our laboratory recently invented a new process of extracting the oil from soybean by the use of alcohol as a solvent and were able to produce, on a semi-industrial scale, soybean cake with a finer appearance than any of the cakes commonly produced, it being devoid of the distasteful qualities above-mentioned. Such physical qualities as well as the high protein content of the product suggest the greatest possibilities of its providing in the near future a new important source of protein in the human dietary.

In this series of investigations we wished to study the nutritive value of soybean alcohol extracted cake as well as the value of its protein to supplement those of common cereals. According to Ishida(2) the soybean cake produced by the alcohol extraction method contains a much larger amount of denatured protein than those obtained by extraction with other solvents like benzine and benzene, and he accordingly suggested that the protein of alcohol extracted cake may not possibly be well utilized by the animal organism. We thought, therefore, that it would be of the utmost importance, in recommending this new kind of soybean cake for human food, to estimate to what extent its protein can be utilized by human beings.

In the first paper we reported on the results of our digestive experiments in which the coefficients of the digestibility of protein supplied by alcohol extracted cake were determined on both white rats and humans.

Experimental Results

Test Materials.

The samples of alcohol extracted and benzine extracted soybean cake flours were supplied by the Experimental Soybean Oil Plant in our laboratory and that of round press-cake was obtained from the Nisshin Oil Mill of Dairen.

The alcohol extracted cake flour used in the experiments was prepared as follows: 40 kg. of soybean were crushed by iron rollers, dried with hot air and heated at about 80°C for 3 hours with 140 L. of ethyl alcohol (95~6 per cent) in a closed rotary extractor. During the extraction a portion of hot alcoholic solution was continuously removed from the extractor and passed through a cooler and after using the separator to take off the dissolved oil it was recirculated into the extractor. At the end of the operation the alcoholic solution was drawn off and the residue remained in the extractor was dried under a reduced pressure with a stream of hot air at 60~70°C. The cake thus obtained was then subjected to a milling machine by which it was ground into a flour and passed through 124 mesh sieves, the majority of skins of soybean being removed by sifting.

The alcohol extracted soybean cake flour as well as other test materials employed in the experiments had the chemical composition as shown in Table I.

Table I.

Kind of Soybean Flour	Moisture %	Ash %	Crude Fat %	Crude Protein %	Nitrogen		H$_2$O-Sol. N. in Total N. %
					Total %	H$_2$O-Soluble %	
Unextracted Soybean	8.4	5 7	17.5	42 5	6.8	6.0	88.3
Alcohol Extracted Cake	8.1	6 2	1 4	56.9	9.1	2.2	24.2
Benzine Extracted Cake	7 6	6 3	1 3	56.2	9 0	7.4	82 2
Round Press-Cake	10 5	5.9	7.4	46 9	7.5	3 4	45.3

Digestive Experiments on Rats.

To each group consisting of 6~8 rats weighing about 100 g. were given 12 experimental diets which contained the proteins supplied by soybean, its alcohol extracted, benzine extracted and pressed cakes at 10, 15 and 20 per cent, together with starch McCollum's salt mixture No. 185, soybean oil, cod liver oil and oryzanin, this being a vitamin B preparation. In preparing the diets these ingredients were thoroughly mixed with water and cooked for about 2 hours in a gas oven.

The experimental period lasted for one week, during which the total nitrogen of rations taken by each group of rats as well as that of feces excreted by them were noted daily to estimate the coefficients of apparent digestibility at different intake levels for the proteins supplied by these test materials. For the calculation of true digestibility some corrections based on the excretion of nitrogen derived from metabolic products and from intestinal bacteria were made, rats weighing about 100 g. being found to have excreted on an average 0.075 g. of nitrogen per week when fed with a nitrogen-free ration.

As indicated in Table II it was shown that the coefficients of apparent digestibility of proteins were much influenced with the amount of proteins ingested by rats while those of true digestibility were almost independent from the protein intake, their average values being estimated as follows: soybean flour 84.8 per cent, alcohol extracted cake flour 85.0 per cent, benzine extracted cake flour 85.7 per cent and press-cake flour 84.4 per cent.

From the comparison of these utilization figures it is evident that the protein of alcohol extracted cake, inspite of its marked denaturation, was digested by rats almost in an equal degree with those supplied by soybean and other kinds of its cakes containing less amounts of denatured proteins.

Table II.

Experimental Diet		Amount of N in Diets consumed	Amount of N in Feces	Coef. of Digestibility	
Test Material	Protein Content			Apparent	True
Unextracted Soybean Flour	10%	5.51 g.	1.267 g	77.0%	85.2%
	15	6.28	1.483	76.4	83.6
	20	8.87	1.732	80.5	85.6
				Average	84.80
Alcohol Extracted Cake Flour	10	7.93	1.692	78.7	84.4
	15	10.51	2.281	78.3	84.0
	20	12.97	2.359	81.8	86.5
				Average	84.97
Benzine Extracted Cake Flour	10	4.58	1.049	77.1	86.9
	15	7.27	1.552	78.7	84.9
	20	12.86	2.498	80.6	85.3
				Average	85.73
Round Press-Cake Flour	10	7.56	1.884	75.1	83.0
	15	8.07	1.598	80.2	85.8
	20	16.07	3.129	80.6	84.3
				Average	84.37

Digestive Experiments on Humans.

Bread and biscuits prepared with the mixtures of wheat flour and alcohol extracted soybean cake flour (20~40 per cent) were given to 4 men for 6 days of experimental period which succeeded the preliminary period of 3 days during which the subjects ate plain bread and biscuits instead of soya-bread and soya-biscuits as principal foods. . During the entire periods they were requested to take definite amounts of accessory foods every day as follows: breakfast, 1 apple; lunch, 90 g. "carnation milk" and 20 g. sugar; dinner, 2 fried eggs and 200 g. the leaf of "hakusai", Brassica Campestris L. Tea and water were to be taken in liberal quantities.

The amount of food proteins taken by individual subject during the experimental period varied from 543.8 to 912.5 g. 41~47 per cent of which were derived from the soybean cake; no one experiencing any physiological abnormalities.

To estimate the coefficients of digestibility for the protein of soybean cake it was assumed that the proteins supplied by wheat flour, egg, milk, hakusai and apple were 88, 97, 94, 85 and 85 per cent digested respectively.

The experimental results were shown in Table III in which we estimated that the protein supplied by soybean alcohol extraction cake was 84.7~91.8, or on an average 88.6 per cent utilized by these subjects. Holmes([illegible]) who fed 7 men with biscuits made with a mixture of wheat flour and soybean press-cake flour together with fruits, butter and sugar calculated that the protein supplied by soybean press-cake was 79.4～90.2 per cent, or approximately 85.3 per cent digested by them.

Table III.

Subject, Name Age Body Weight	C K.T. 21 54.0 kg.		C K.M. 15 35 5 kg.		C C R. 18 57.5 kg.		S I. 33 53.5 kg.	
Foods consumed	Weight of Food	N in Food	Weight of Food	N in Food	Weight of Food	N in Food	Weight of Food	N in Food
	g.	g.	g.	g.	g.	g	g.	g
Soya-Bread	4930	105.98	2802	60.24	4512	97.01	2481	53.34
Soya-Biscuits	796	18.23	718	16.44	1206	27 62	552	12.64
Apple	713	0.28	780	0.31	777	0.31	625	0.25
Egg	585	11.45	637	12.46	627	12 28	618	12.12
Butter	420	0 67	240	0.38	420	0.67	240	0 38
Carnation Milk	540	5.94	540	5.94	540	5.94	540	5 94
Sugar	120	—	120	—	120	—	120	—
Leaf of Hakusai	1200	2.40	600	1.20	1200	2.40	1200	2.40
Total	9304	144.95	6437	96 97	9402	146.23	6376	87 07
Calorie Intake per day (average)	3419 Cal		2330 Cal.		3535 Cal		2101 Cal.	
Feces excreted								
Total Solid Matter	187 0 g		154.0 g		263 0 g.		112 0 g.	
Total Nitrogen	14 00		10 63		18.39		7.70	
N derived from undigested soybean protein	5.94		5 52		10.51		2.97	
Coef. of Digestibility								
Total Food Protein	90 34%		89.00%		87 42%		91.15%	
Average	—		—		—		89.48	
Soybean Protein	91.07		86 96		84.71		91 77	
Average	—		—		—		88.62	

Based on the comparison between these utilization figures it may be justified to conclude that the protein of soybean alcohol extraction cake is comparatively well digestible by men, its coefficient of digestibility being just as large as that of protein supplied by soybean press-cake.

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II. On the Nutritive Value of Soybean Alcohol Extracted Cake.

By

Seiichi Izume, Yoshinori Yoshimaru and Isao Komatsubara.

Introduction

The facts that soybean contains a large amount of physiologically good protein and is rich in the water-soluble growth factor were first demonstrated by Daniels and Nichols(1), and Osborne and Mendel(2) and were subsequently observed by a number of investigators.

It was also shown by Osborne and Mendel that the protein supplied by soybean press-cake was not inferior in the nutritive value to that of soybean. According to Ohtomo(3) the protein supplied by soybean benzine extracted cake was noticeably inferior in the physiological value to that derived from press-cake. He also demonstrated that the nutritive value of soybean cakes was decreased to certain extents by treating them with hot alcohol; he ascribed these facts to the denaturation of soybean protein caused by the application of the solvent and heat. His conclusion were, however, not very convincing, being but poorly supported by the results of his feeding experiments.

In the present investigations we compared the nutritive value of the protein of alcohol extracted cake with those supplied by soybean, its benzine extracted and pressed cakes and tried to ascertain whether the denaturation of protein was the determining factor in the evaluation of nutritive efficiency of soybean protein. We also made comparisons between soybean and its alcohol extracted cake for the values in furnishing essential vitamins.

Experimental Results

The Nutritive Value of Protein Supplied by Soybean Alcohol Extracted Cake Flour.

In evaluating the nutritive value of protein supplied by soybean alcohol extracted cake young rats weighing about 40~50 g. were placed on experimental diets for periods varying from 140 to 240 days, and the rates of their growth, fertilities of female rats as well as the abilities of mothers to rear their young were carefully observed. The diets contained the alcohol extracted cake as the sole source of protein which supplied 7.5, 10, 15 and 20 per cent protein and were supplemented with other essential nutrients, such as, starch, McCollum's salt mixture (5%), cod liver oil (3%), soybean oil (7%) and oryzanin (5 c.c. per 100 g. of diet).

The rats, when fed upon a diet (No. ACMa) including the protein supplied by alcohol extracted cake at 7.5 per cent, grew at a slow rate; their body weights seldom reaching 150 g. in 140 days of the experimental period, and of which none of the females successfully reproduced. With a diet (No. ACMb) containing protein at 10 per cent, rats acquired a better growth, but their growth curves were decidedly inferior to those of normal nutrition observed by Osborne and Mendel. No offsprings were observed to have been produced on this diet. When the protein content of diet was increased to 15 per cent (Diet No. ACMc) the growth rates were much improved; the females often producing the young but failing to suckle them successfully in most cases, but when the protein formed 20 per cent of diet (No. ACMd) rats secured a normal growth and the females produced normal litters of young which were normally suckled. The growth curves shown by rats placed on these four kinds of experimental diets are indicated in Figure I.

We also conducted a number of feeding experiments under the otherwise similar conditions employing soybean flour, soybean benzine extracted and pressed cake flours as test materials. The diets contained the proteins supplied by these materials at 7.5, 10, 15 and 20 per cent, together with other nutrients similar to those employed in the previous experiments. The experimental results demonstrated that these proteins of different preparations with their various contents of denaturated proteins were very similar in their nutritive values to that of soybean alcohol extracted cake, when employed as the sole source of protein.

These facts prove that the physiological value of soybean protein is not significantly altered by heating it under the described conditions with such solvents as alcohol or petroleum benzine, although alcohol causes a marked physical change (denaturation) of protein. Our results also confirm those of previous investigators who have demonstrated that soybean contains protein of a high physiological value.

Comparison of the Vitamin A Value between Soybean and its Alcohol Extracted Cake.

The fact that the addition to diets of soybean protein at 20 per cent supplied a sufficient amount of protein for a normal growth of rats induced us to use soybean and its alcohol extracted cake as a means of suppling both vitamin A and protein in the diets for the evaluation of the vitamin A factor of these test materials.

One diet consisted of 47 g. of soybean flour which supplied 20 per cent protein with 45 g. of starch, 5 g. of McCollum's salt mixture, 3 c.c. of irradiated ergosterol solution (0.001 per cent in olive oil) and 5 c.c. of oryzanin. Two other diets were composed of 35 and 53 g. of alcohol extracted cake flour which furnished 20 and 30 per cent protein, 50 and 32 g. of starch and 7 and 7 g. of soybean oil, besides the same amounts of inorganic salts, irradiated ergosterol and oryzanin as were contained in the former diet.

Young rats showed when placed on the diet which was 47 per cent soybean a fairly rapid gain of weight at an initial period of 3~5 weeks which was followed by a retardation and cessation of growth, after which the animals finally died as they developed ophthalmia due to the lack of vitamin

A. The addition to the diet of 3 per cent of cod liver oil, however, was followed by a prompt recovery from the eye disease and an immediate resumption of growth, the fact proving that soybean flour was not great in the vitamin A value.

On the diets in which soybean alcohol extracted cake flour was added at 35~53 per cent the majority of rats showed a less rapid growth at the beginning and developed ophthalmia, dying sooner than those on the previous diet.

These experimental results indicate that while the vitamin A values of both soybean and its alcohol extracted cake are not considerably great, the latter is undoubtedly inferior in the content of this factor.

Comparison of the Vitamin B Value.

Our procedure to evaluate the vitamin B factor of soybean and its alcohol extracted cake involved the feeding of young rats on diets in which the test materials were incorporated as the sole sources of both vitamin B complex and protein, and observing their power to promote the growth of animals. The experimental diets contained 47 per cent soybean or 35~53 per cent alcohol extracted cake and were equally supplemented with starch, McCollum's salt mixture (5%) and cod liver oil (3%), their protein contents varying from 20 to 30 per cent.

Two specimens of alcohol extracted cake were tested for the vitamin potency. One of them "No. A" was obtained from soybean by extraction with fresh alcohol and the other one "No. B" by treating it with the alcoholic solution which had been repeatedly used in the previous extraction processes for more than ten times.

The rats placed on the diets containing 47 per cent soybean as well as those on the ration 53 per cent of which was alcohol extracted cake "No. B" were capable of attaining normal growth, while those on the diets including alcohol extracted cake "No. A" at 35~53 per cent were unable to secure a good growth, their growth rates being much inferior to those of normal nutrition.

Conclusions may be drawn from these results that while soybean contains a fairly abundant quantity of vitamin B, the value of alcohol extracted cake for this factor is dependent on the kind of solvents used in the extraction; the cake obtained by means of fresh alcohol is not great in this essential while the one produced by extraction with the alcoholic solution which had been repeatedly used in the previous extraction is shown to be quite potent in this factor, its value being not much different from that of soybean.

Comparison of the Vitamin D Value.

Young rats were placed on the experimental diets for 4 weeks and at the end of the period the rachitic changes produced at the distal end of the tibia were radiographically examined. The diets consisted of 24～47 per cent soybean or of 18～53 per cent its alcohol extracted cake, besides starch, butter (5%), calcium carbonate (3%), sodium chloride (2%) and oryzanin. The control animals while placed on the same diets were daily fed with 1/100 mg. of irradiated ergosterol.

The results of experiments showed that while the control animals produced no rachitic lesions all the rats placed on the diets containing soybean at 24～48 per cent as well as those on the diets including 18～53 pre cent alcohol extracted cake were demonstrated to have been afflicted with rickets, the fact indicating that both soybean and its alcohol extracted cake have comparatively little vitamin D value.

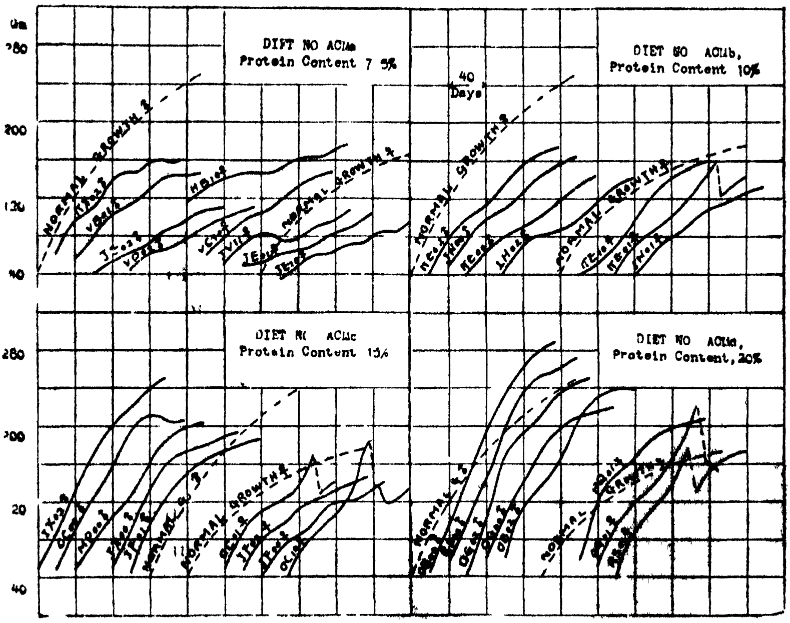

Fig I The growth curves of rats placed on diets containing various amounts of protein supplied by alcohol extracted soybean cake as the sole source of protein.

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III. Supplementary Dietary Relations of the Protein of Soybean Cake for those of Cereals.

By

Seiichi IZUME and Isao KOMATSUBARA.

Introduction

Such cereals as wheat, rice, maize and kaoliang, commonly used as human foods, are relatively poor in the protein content and their proteins are more or less incomplete in satisfying the physiological requirements of the animal organism, being deficient in some essential amino-acids, especially in lysine. Soybean and its cake, on the other hand, contain a large amount of proteins which are comparatively rich in lysine. It is, therefore, naturally expected that the addition of soybean or its cake to the above cereals might elevate the nutritive values of cereals by increasing their protein contents and by improving the qualities of their proteins.

It was already demonstrated by Osborne and Mendel(1), McCollum, Simmonds and Parsons(2) and Johns and Finks(3) that the protein of soybean was capable of supplementing efficiently those of wheat and maize proteins. The present investigations were undertaken with the intention of demonstrating as to what extent the mixtures of soybean alcohol extracted cake flour with the flours of these cereals were superior in the nutritive value to those of cereal flours alone, provided adequate amounts of various vitamins and inorganic salts were similarly supplemented. The values of the protein supplied by alcohol extracted cake in supplementing the proteins of cereals were also studied.

Experimental Results

Test Materials.

The samples of soybean alcohol extracted cake, wheat, rice (polished), maize (entire kernel) and kaoliang (unpolished) flours employed in these experiments had the chemical composition as indicated in Table I.

Table I.

Kind of Flour	Moisture (%)	Crude Protein (%)	Ash (%)
Wheat No. A.	9.7	10.6	0.59
Wheat No B	10.1	13.5	0.47
Rice	11.8	6.9	0,33
Maize	12.5	7.0	1.20
Kaoliang	10.8	9.8	1,77
Soybean Cake	8.1	56.9	6.20

Nutritive Value of the Wheat Flour and Soybean Cake Flour Mixture.

The young rats which were placed on a diet (No. F) consisting of wheat flour (85%) McCollum's salt mixture (5%), cod liver oil (3%), soybean oil (7%) and oryzanin (5 c.c. per 100 g. diet) failed to accomplish a good growth and all the female rate remained sterile. The animals, however, attained a normal growth when fed upon adiet (No. FACM5) composed of a 95 percent wheat flour and 5 percent soybean cake mixture, together with inorganic salts, cod liver oil, soybean oil and oryzanin similarly supplemented as in the previous diets. Female rats produced normal litters of young and suckled them successfully in most cases. The brood of the second generation placed on the diet the same as that on which their parents were raised made a fairly good growth.

Animals also secured an excellent growth, by two kinds of diets (No. FACM10 and No. FACM20) which contained the wheat flour 10 and 20 percent of which had been replaced with soybean cake flour and were similarly supplemented with other nutrients, their growth rates being similar to or even better than those of normal nutrition. Several broods of vigorous young were produced which were successfully suckled by mothers in most cases and grew normally on the same diets as those of their parents.

The growth curves of rats placed on these four diets are compared in Figure I.

Nutritive Values of the Mixtures of Soybean Cake Flour and other Cereal Flours.

The results of our experiments demonstrated that the feeding of rats with rice, maize or kaoliang flour together with adequate amounts of inorganic salts and vitamins failed to induce in them a satisfactory growth, but when soybean cake flour was added to rice or maize flour at 10 per cent and to kaoliang flour at 20 per cent the mixtures were found to supply enough protein for the normal growth of white rats.

Value of Protein of Soybean Cake to Supplement That of Wheat Flour.

Wheat flour "No. B" with a higher protein content was added with Mc Collum's salt mixture (5%), cod liver oil (3%), soybean oil (7%), oryzanin and starch to bring the protein content of diet up to a 10 per cent level. Another diet was prepared with the mixture of wheat flour and soybean cake flour (80 : 20) and similarly supplemented with other elements, it containing protein at 10 per cent, one half of which was furnished by wheat and another half by soybean cake.

Two groups of young rats were placed on these diets and their growth rates were compared with each other and also with those of rats on the diet in which the protein supplied by soybean cake was included at the same level. These comparisons showed that at a 10 per cent level of protein intake the mixed proteins derived from wheat flour (5%) and soybean cake (5%) were much greater in nutritive efficiency than the protein of soybean cake or of wheat flour supplied as the sole source.

These observations are in harmony with those of McCollum, Simmonds and Parsons(2) who found that rats fed on a diet containing mixed proteins at 9 per cent (6 per cent from wheat and 3 per cent from soybean) grew much more rapidly than those on diets containing the same amount of protein derived from either wheat or soybean alone. We attributed these facts to the effects of soybean protein to supplementing the deficiencies in essential amino-acids of wheat protein, especially in lysine and arginine.

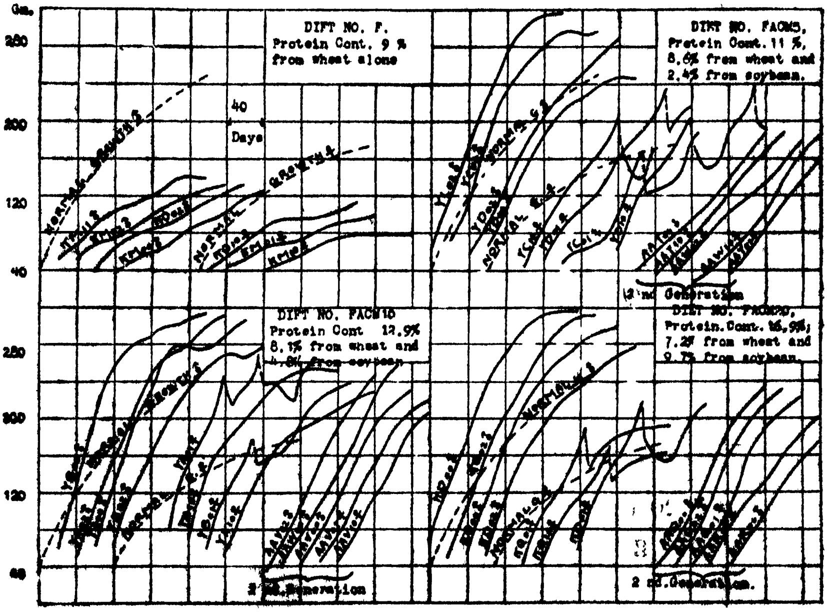

Fig. I. The growth curves of rats showing the differences in the nutritive value between wheat flour and the mixtures consisting of wheat flour and alcohol extracted cake soybean flour.

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The Influence of Ultra-violet Rays upon the Hatching Quality of Eggs.

By

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(Imperial Zootechnical Experiment Station, Chiba, Japan)

(Received July 12, 1931)

Groups of eggs were exposed to the radiation of a "Silectra-Standard" quartz mercury-vapor lamp provided with a filter in periods varying from 15 minutes to 3 hours at a distance of 40 cm. from the lamp. In addition, groups of eggs unexposed to ultra-violet rays were incubated with those that had been exposed, and the following results were obtained.

1. The direct exposure of eggs to ultra-violet rays, whether irradiated just before placing them in the incubator or during the incubation processes, had no influence on the hatching quality of the eggs, whether white-shell or brown-shell eggs.
2. The exposure of the hen herself to ultra-violet rays will improve markedly the hatching quality of eggs, but eggs which are laied after the irradiation of the hen has been suspensed are not influenced by the previous irradiation.
3. The wave length which can penetrate through the egg-shell measures about 3100 Å, but the egg-shell with the shell-membrane is opaque to radiation shorter than 3660 Å in wave length.

The Influence of Ultra-violet Rays upon the Production of the Egg.

By

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(Received July 12, 1931)

In order to ascertain the influence of the irradiation of ultra-violet rays on egg production in the winter season under the usual management of laying-hens, 36 pedigreed single comb white leghorn hens were selected and divided into two groups as uniformly in reference to laying history and breeding as could be done and each group was consisted of five 5-year-old hens, three 3-year-old hens and ten pullets just begun laying.

These groups of hens were housed in two pens of the same construction and adjoining each other, and a male bird was placed in each of the two pens.

The following ration was given to both groups.

Mash	Wheat bran	150 parts
	Rice bran	150 〃
	Barley bran	80 〃
	Soybean cake	70 〃
	Fish meal	50 〃
Grain	Yellow corn	300 〃
	Wheat	200 〃

The ultra-violet light used was radiated by an "Acme Jesioneck" quartz mercury-vapor lamp. One group of hens was irradiated as a group 30 minutes daily at a distance of 1~2 meters for 120 days from November 1, 1928, to February 28, 1929; the other group was not irradiated.

We compared the number of eggs which were produced by both groups of hens during the above mentioned experimental period and further for 92 days after the suspension of the irradiation, from March 1 to May 31, 1929.

The following results were obtained:

1. The pullets, which had just begun laying, irradiated by ultra-violet rays produced more eggs than the non-irradiated ones, but the old hens, from 3 to 5 years old, were not consistently affected by the irradiation.
2. There was no noticeably bad effect upon the egg-laying after the suspension of the irradiation of ultra-violet rays.
3. The average daily food consumption per hen of both groups, irradiated and non-irradiated, was almost the same.

A New Method for the Estimation of Uric Acid in Poultry Excrement.

By

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(Received July 12, 1931)

1 g. of the dried and powdered urono-fecal mixture of poultry excrement is weighed into a beaker of 100 c.c. capacity and 20 c.c. of distilled water added. The contents of the beaker are now throughly mixed by stirring and brought gently to the boil on the sand bath. After boiling for about 1 minute, 5 c.c. of concentrated hydrochloric acid are added and the boiling

and stirring is continued further for a few seconds in order to convert the urates in the excrement into free uric acid and chlorides. The beaker is now set aside overnight in a cold place. The acid liquid is filtered off through a small filter paper and the residue on the filter paper is washed with distilled water which has been cooled with ice previously, until the washings contain no trace of hydrochloric acid and then treated with 90 per cent alcohol and ether successively in order to remove free fatty acids. Both the residue and the filter paper altogether are once more returned into the beaker and 20 c.c. of distilled water added. The contents of the beaker are again throughly mixed by stirring and brought to the boil on the sand bath. After boiling for some minutes, 5 c.c. of conc. ammonia liquid are stirred in and the boiling and stirring is continued further for a few seconds. The beaker is then allowed to stand for about 1 hour in a room temperature.

The free uric acid is converted into mono-ammonium urate by this treatment. The ammoniacal liquid in the beaker is now evaporated to dryness on the water bath and then the beaker is placed in the steam oven and heated at 100°C for some hours in order to remove the excess of ammonia completely. 20 c.c. of 5 per cent hydrochloric acid are now poured in the beaker and the contents of the beaker are again throughly mixed by stirring and boiled gently for about 1 minute on the sand bath. By this treatment, mono-ammonium urate is decomposed to the free uric acid and ammonium chloride.

The beaker is allowed to cool for some hours in order to crystallize out the uric acid completely. The acid liquid is now filtered off through the small filter paper and the residue on the filter is washed with cold distilled water which has been cooled with ice previously, till perfectly free from chloride.

The filtrate and washings are evaporated to about 10 c.c. on the water bath and then made up exactly 100 c c. with absolute alcohol. 50 c.c. of the alcoholic solution are pippetted in the flask of a steam distilling apparatus and the excess of hydrochloric acid is neutralized with dilute alcoholic potash using phenolphthalein as an indicator and the 2 or 3 drops of the alcoholic potash are added to make the liquid faint alkaline.

The flask is connected with condencer and a rapid current of steam derived from distilled water which has been freed from CO_2 by prolonged boiling, is passed through the solution until a foam nearly filling the flask develops.

All ammonia in the solutions is distilled into a measured quantity of 1/5 or 1/10 normal solution of sulfuric acid. The excess of acid in the alcoholic contents of the receiver is determined by titrating with 1/10 normal solution of NaOH or KOH using alizarin (0.5 per cent alcoholic solution) as an indicator.

The amount of nitrogen of the distilled ammonia is estimated by the amount of alkali equivalent to the distilled ammonia.

In calculating the amount of uric acid, the amount of the estimated nitrogen may be multiplied by the factor 11.998 which is the ratio of a molecular weight of uric acid and an atomic weight of nitrogen.

The accuracy of this method for estimating uric acid in poultry excrement was confirmed by the following experiments.

Experiment I. A known amount of pure uric acid (Kahlbaum) was taken and estimated its quantity by our new method, and the following result was obtained.

Weight of pure uric acid taken	0.2989 g.
Amount of uric acid analysed	0.2985 g.
Error	0.13%

Experiment II. 0.5 g. of dried and powdered poultry urine which was collected separating from the feces by means of an artificial anus, was taken and its uric acid content was estimated. Then, a known amount of pure uric acid was added to 0.5 g. of the dried urine and total amount of uric acid was determined, and the quantity of uric acid orginally added was calculated. The following result was obtained.

Weight of pure uric acid added	0.09963 g
Amount of uric acid determined by analysis	0.09916 g.
Difference	0.00047 g.
Error	0.472%

Experiment III.

(A) 1.0 g. of dried and powdered poultry feces which was collected by means of a artificial anus and free from urinary constituents, was taken and treated to carry out the estimation of uric acid by our new method. We have obtained such result as the feces contained 0.336 per cent uric acid. Weighed amount of pure uric acid was submitted to the complete series of processes involved in the method and the following satisfactory result was obtained.

	(a)	(b)
Weight of pure uric acid added	0.09963 g.	0.1926 g.
Amount of uric acid analysed	0.09950 g.	0.20001 g
Percentages of recovered amount	99.870%	100.376%
Error	0.130%	0.370%

(B) The feces which was used in Experiment III, (A) ought to contain no uric acid, but owing to the result of the analysis there existed 0.336 per cent uric acid in it. In order to ascertain definitely whether the incompleteness of the separation of urine and feces was due to obtain such result or the existence of certain volatile basic substances which were distilled by the steam distillation, interfered with the accuracy of the method, the bird whose feces was used in Experiment III (A), was killed and the content of the small intestine was dried and then the uric acid estimation was carried out by our method. It resulted to show that the dried content of the small intestine contained 0.386 per cent uric acid.

It can be said by this experiment that some volatile basic substances in poultry feces are distilled by the steam distillation, but its amount is negligible.

Experiment IV. One bird (S. C. White Leghorn No. 3) was fed with soybean cake alone (high protein ration) and the other two (S. C. White Leghorn No. 1 and No. 2) were fed with kaoliang alone (low protein ration). Their excrements were collected separately, and the uric acid contents were determined by our method. The agreement between the duplicates, (a) and (b), in the trials is shown by the following figures.

		(a)	(b)
Uric acid content of the excrement of bird	No. 1	8.168%	7.832%
〃	No. 2	7.008%	6.840%
〃	No 3	26.27%	26.27%

Experiment V. Weighed amount of the pure uric acid was added to each excrement of the birds, which was used in Experiment IV, and total amount of uric acid was determined. Using the figures obtained in Experiment IV, the amount of uric acid orginally added was calculated and satisfactory results were obtained, as shown by the following figures.

	Weight of uric acid added (g.)	Amount of uric acid determined by analysis (g)	Difference (g.)	Error (%)
Experiment of No 1	0.1993	0.19832	0.00098	0.161
No. 2	0.1993	0.19826	0.00104	0.572
No. 3 (a)	0.1993	0.19898	0.00032	0.161
(b)	0.1494	0.14991	0.00051	0.341

Studies on the Fermentation Products by Mould Fungi. Part IX.

By

Yusuke SUMIKI.

(Agricultural Chemical Laboratory, Tokyo Imperial University)

(Received June 2, 1931)

In parts IV and VIII of this series (Agr. Chem. Soc. Japan, 5, 10, 1929; 6, 106, 1930) it was shown that the 2-oxymethylfuran-5-carboxylic acid was formed by Asp. glaucus under the particular cultural condition and the mechanism of the formation of this acid was cleared. In this paper, 15 kinds of Aspergillus have now examined whether to produce the 2–oxymethylfuran–5–carboxylic acid under this particular cultural condition and it comes to know that clavatus, glaucus, niger, oryzae, wentii produce this acid. Besides the formation of 2–oxymetylfuran–5–carboxylic acid, the interesting fact is the formation of a considerable amount of mannite by Asp. clavatus. As other fermentation products, ethyl alcohol, acetaldehyde, succinic, fumaric, oxalic, gluconic and acetic–acids are isolated and identified.

The fermentation products of every fungus are as follows.

Asp. candidus: Ethylalcohol, acetaldehyde, succinic acid, acetic acid.
Asp. clavatus: Ethylalcohol, acetaldehyde, succinic acid, 2–oxymethylfuran–5–carboxylic acid, mannite.
Asp. fisheri: Acetaldehyde, succinic acid, gluconic acid.
Asp. flavus: Ethylalcohol, acetaldehyde, fumaric acid.
Asp fumigatus: Ethylalcohol, acetaldehyde, succinic acid, acetic acid
Asp. glaucus: 2–oxymethylfuran–5–carboxylic acid, gluconic acid.
Asp. niger: Ethylalcohol, acetaldehyde, succinic acid, 2–oxymethylfuran–5–carboxylic acid.
Asp. oniki: Acetic acid, fumaric acid
Asp oryzae T. N.: Ethylalcohol, acetaldehyde, 2–oxymethylfuran–5–carboxylic acid.
Asp. ostianus: Ethylalcohol, acetaldehyde, succinic acid, gluconic acid.
Asp pulverentus: Ethylalcohol, acetaldehyde, oxalic acid, gluconic acid.
Asp. sydowi: Ethylalcohol, acetaldehyde.
Asp. terreus: Ethylalcohol, acetaldehyde, succinic acid.
Asp. versicolor: Ethylalcohol, acetaldehyde, acetic acid.
Asp wentii: 2–oxymethylfuran–5–carboxylic acid, fumaric acid.

Über die physiologischen Wirkung der Phytosterin-ester.

Von

Zirō NIKUNI.

(Eingegangen Juni 8, 1931)

Zur Erklarung der physiologischen Wirkung der Phytosterin–ester machte der Autor das vergleichenden Tierexperiment zwischen den Cholesterin– und Phytosterin–estern (Acetat und Palmitat).

Die Resultate sind folgend:

1. Zur Ernährung der weissen Ratten hat Phytosterin–ester sowie Cholesterin–ester die ansehenlichen Schädigungskraft nicht. Und zwischen den beiden Sterinen ist kein klarer Unterschied. Aber im allgemeinen steht der Acetat der beiden Sterine dem Palmitat an der Ernährung der Tiere nach.

2. Die Sterin–ester, die von Munde gegeben sind, ausscheiden etwa die

Hälfte der Menge im Kot als die gegebenden Formen. Die Ausscheidigungsmenge der Phytosterin-ester ist geling wenig als die der Cholesterin-ester. Im allgemeinen ist die Ausscheidigungsmenge der Palmitat grösser als die der Acetat.

3. Der Sterin-gehalt der experimentierten Tiere ist grösste bei der mit Cholesterin-ester gefütterten Tieren. Der Steringehalt, der mit Phytosterin-ester gefütterten Tiere ist doch grösser als der der kontrollen Tiere.

4. Das Körpersterin der mit Phytosterin-ester gefütterten Tiere enthält die kleinen Menge des Phytosterins.

Der Schluss.

Das Phytosterin, das mit dem Nahrungsmittel gegeben ist, ist ein Teil absorbiert und setzt sich im Tierkörper wie das Cholesterin.

Und die absorbierenden und setzenden Grade der beiden Sterine sind zu ihren Esterformen abhangig.

Chemische Untersuchungen über das Glukomannan aus "Konjak".

V. Mitteilung. Methylierung des Glukomannans.

Von

Kitsuji NISHIDA und Hideo HASHIMA

(Eingegangen am 26 Juni 1931)

Der Methylierungsversuch gibt die Aufklärung über das Konstitutionsproblem des Glukomannans und durch ihn wird ein gewisser eindeutiger Abschluss erreicht. Zunächst wurden 10 g. Glukomannan mit 200 ccm. 15%-iger Natronlauge und 96 g. Dimethylsulfat methyliert. Nach 6–mal Methylierung betrug der Methoxylgehalt 34 %. Durch weitere Methylierungen, unter Verwendung von 20 g. Glukomannan, 30 ccm. 30%iger Natronlauge und 288 g. Dimethylsulfat für jeden Methylierungsversuch, war dann der Methoxylgehalt ein höhere geworden und 12∼14 malige Methylierung diesen Wert 41.5% hatte erreichen lassen.

Bei der Methylierung von 2 g. methylierten Glukomannans (41.6% Methoxyl) mit 50 ccm. Methyljodid und 36 g. Silberoxyd erreichte der Wert nicht über 41.6% OCH_3. Wir mussen hieraus den Schluss ziehen, dass das Glukomannan bei der Methylierung mit Dimethylsulfat und Alkali oder mit Methyljodid und Silberoxyd gleichviel Methoxyl liefert wie die Cellulose und das Lichenin, d. h. dass er nicht uber 42% zunehmen kann, und dass die Methylierung beim Arbeiten mit 30%iger Natronlauge und bei niedrigerer Temperatur leichter ist.

Das Methylglukomannan (OCH_3 41.6%) ist in kaltem Wasser kolloidal lösbar, in heissem Wasser dagegen tritt wieder Ausflockung ein, wie der Methylather der Cellulose, des Lichenin und der Starke. Es löst sich in Chloroform, Bromoform, Alkohol, Aceton, Eisessig und Essigester, doch haben wir eine Molekulargewichtsbestimmung nicht ausgeführt.

Die Hydrolyse des Glukomannanathers führt zu methylierten Hexosen, und hat keine Abspaltung von Methylgruppen im Gefolge. Zu diesem Zweck wurden 29.8517 g. methyliertes Glukomannan (OCH_3 41.5 %) mit 1%iger methylalkoholischer Salzsäure in Bonbenröhren zu den methylierten Methylglukosiden und –mannosiden aufgespalten. Das Spaltungsprodukt wurde mit Silberkarbonat neutralisiert und es wurden nach dem Trocknen im Hochvakuum bei der fraktionierten Destillation fünf Fraktionen aufgefangen. Die ersten drei Fraktionen zeigten ein fast farbloses, die späteren ein schwach gelbliches Aussehen, und alle färbten beim Stehen in langer Zeit nach. Die Analyse ergaben.

	Sdp. (°C)	OCH$_3$ %		n_D^{15}	$[\alpha]_D^{20}$	
					in Wasser	in Methylalkohol
I Fraktion	124~125	50.83	50.11	—	—	—
II. Fraktion	125~127	51.10	50.74	1.4604	+43.8°	+45.6°
III. Fraktion	127~129	48.68	48.11	1.4587	+42.3°	+52.8°
IV-A. Fraktion	126~128	47.96	48.57	1 4597	+43.0° +42.5°	—
IV-B. Fraktion	128~139	47.16	47.08	1.4626	+45.9°	+56.6°

Aus diesen Versuchen geht also hervor, dass die Konstanten der Fraktionen, die aus methyliertem Glukomannan einerseits, aus Methylcellulose und -lichenin anderseits durch Spaltung gewonnen worden sind, eine voreinander erheblich abweichende Zusammensetzung zu haben scheinen, da das Glukomannan aus 2 Mol Mannose und 1 Mol Glukose besteht und die Spaltprodukte müssen Trimethylmannosid und -glukosid enthalten.

Die I. und II. Fraktion stehen im Methoxylgehalt ca. 1.1% unter denjenigen, welche sich für ein Trimethylmethylmannosid oder -glukosid aussprechen lassen wurden (52.6%); es muss darin kleine Mengen Dimethylmethylmannosid oder -glukosid (41.8%), das in der III.-IV. Fraktion überwiegt, enthalten sein. Der Methoxylgehalt der II. Fraktion war sehr gleichmassig, aber Brechungsindex war etwas hoch, und die spezifische Drehung war klein, verglichen nicht mit denjenigen, die bei Methylcellulose und -lichenin durch Abbau erhalten werden.

Die II. Fraktion wurde mit wasseriger Salzsaure verseift und dabei schied sich nicht die kristallisierte 2, 3, 6-Trimethylglukose (41.89% OCH$_3$) nach langem Stehen wie diejenige, die aus der ersten Fraktion von Methylbaumwolle und Methylolichenin abgebaut wurde, ab. Dieser Sirup erwies:

%OCH$_3$ 40.49 ; 40.49.

Es muss darin eine Spur Dimethylhexose (29.81% OCH$_3$) enthalten sein. Wir haben diesen Sirup mit Salpetersaure (spez. Gew. 1.20) oxydiert und erhielten Trimethylzuckersaurelakton oder -mannozuckersaurelakton mit folgenden Eigenschaften:

OCH$_3$-Gehalt gef.	37.15; 36.89%
Berechnet für Trimethylzuckersäurelakton	39.74%
Verbrauch von *N*/10-Natronlauge.	10.00 ccm. (Substanz 0,116 g.)

Aus diesem Ergebnis geht also hervor, dass die I. und II. Fraktion 2, 3, 4-Trimethylhexosid waren mit kleine Mengen Dimethylhexosid und nicht 2, 3, 6-Trimethylmethylhexosid, und mit grosser Wahrscheinlichkeit handelte es sich um 2, 3, 4-Trimethylglukosid und nicht Mannosid. Die III, IV-A und -B Fraktion wurden auch wie die erste Fraktion mit Salzsaure verseift, dabei wir Trimethylhexose erhielten, die nach langem Stehen keine Kristalle abschied. Die Analyse ergab:

Fraktion	$\%OCH_3$		$[\alpha]_D^{20}$
III.	38.04	38.01	+36° in Wasser
IV-A	38.73	38.46	+18.5° in Chloroform
IV-B	36.26	36.38	+11.6° in Chloroform

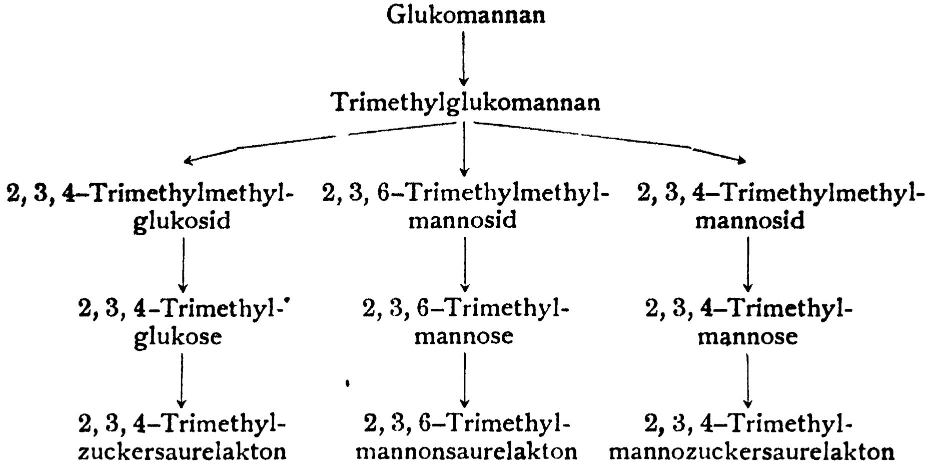
Im Methoxylgehalt stehen diese Fraktionen ca. 3,3∼5.6% unter der Trimethylhexose; es muss darin bereits geringe Mengen Dimethylhexose enthalten sein. Dies Fraktion wurden wie oben mit Salpetersaure oxydiert, und dabei wurden Mono- und Dikarbonsaurelakton mit folgenden Eigenschaften gewonnen.

Aus der III Fraktion OCH_3-Gehalt gef. 36.13; 35.78
Aus der IV-A Fraktion OCH_3-Gehalt gef. 35.18; 35.28
Aus der IV-B Fraktion OCH_3-Gehalt gef. 33.89; 33.76

Fraktion	Substanz	Verbrauch von $N/10$-Natronlauge	Fraktion	Substanz	Verbrauch von $N/10$-Natronlauge
III. Fraktion	0.1071 g	7.52 ccm	IV-B Fraktion	0.1180	9.16
IV-A Fraktion	0.1762	9.98	IV-B Fraktion	0.2631	19.33

Aus hier nahm in der III. und der IV–B Fraktion die Aciditat zu, wahrend der Sauregehalt in der IV–A Fraktion zur Halfte zurückging. Bei all diesem Saurelakton handelte es sich selbstverstandlich um Mischungen, aus denen die erste (aus der III Fraktion) eine Lösung von Trimethylzuckersaurelakton mit kleinen Mengen Trimethylmannonsaurelakton, die zweite (aus der IV–A Fraktion) ein Sirup von Trimethylmannonsaurelakton mit einer Spur Tri- und Dimethylmannozuckersürelakton, die dritte (aus der IV–B Fraktion) eine Mischung von Tri- und Dimethylmannozuckersäurelakton waren. Bei 2, 3, 6–Trimethylmethylglukosid Fraktion finden sich nach Verseifung mit Salzsäure bekanntlich Kristalle von 2, 3, 6–Trimethylglukose, die mit Salpetersäure zu 2, 3, 6–Trimethylglukonsäurelakton (Monokarbonsäurelakton) oxydieren müssen. Der grossere Teil aus der IV–A Fraktion war 2, 3, 6–Trimethylmethylhexosid, aber seine Eigenschaften und das Ergebnis ihrer Analyse zeigte nicht so grosse Aehnlichkeit, dass es sich hier um 2, 3, 6–Trimethylmethylglukosid handeln konnte; der Versuch, in welchem aus methyliertem Glukomannan durch Spaltung keine Kristalle gewonnen wurden, bildete also auch eine Stütze für die Abwesenheit der 2, 3, 6-Trimethylglukose. Da die Trimethylglukose und -mannose mit Phenylhydrazin kein Phenylosazon liefert, so muss am Kohlenstoffatom 2 eine Methoxylgruppe sitzen. Als Gesamtergebnis der im vorstehenden skizzierten Untersuchungen auf dem

Gebiete des Methylglukomannans folgt, dass das Spaltprodukt auf die Weise gewonnen wurde, wie sie das nachstehende Schema zeigt:


Glukomannan
Trimethylglukomannan
2, 3, 4–Trimethylmethylglukosid
2, 3, 6–Trimethylmethylmannosid
2, 3, 4–Trimethylmethylmannosid
2, 3, 4–Trimethylglukose
2, 3, 6–Trimethylmannose
2, 3, 4–Trimethylmannose
2, 3, 4–Trimethylzuckersaurelakton
2, 3, 6–Trimethylmannonsaurelakton
2, 3, 4–Trimethylmannozuckersaurelakton

(Forstchem Institut, Kiushu Kaiserl. Univ.)

Untersuchung über das ätherische Oel aus *Podocarpus macrophylla Don.* III.

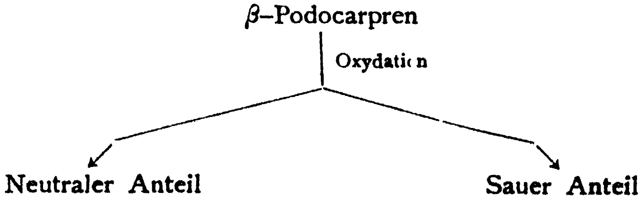
Mitteilung. Ueber die oxydierenden Produkten der neuen Diterpene (α– und β–Podocarpren).

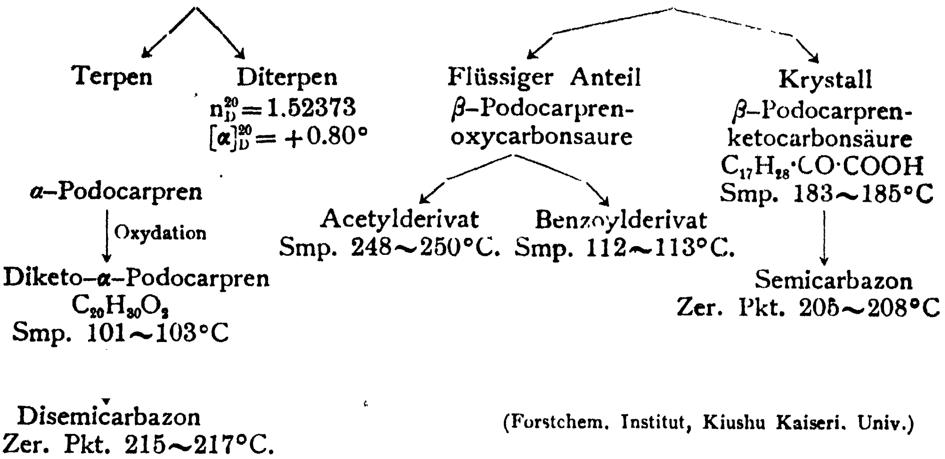
Von

Kitsuji Nishida und Hidetaka Uota

(Eingegangen am 26 Juni 1931)

Zur Konstitutionsaufklarung des α– und β–Podocarpren führten wir den oxdierenden Abbau der Diterpene (1) mit Kaliumpermanganatlösung (2) mit Braunstein und Schwefelsaure (3) mit Kaliumbichromat und Schwefelsäure (4) mit Kaliumpermaganatlösung bei Gegenwart von Alkali durch. Es wurde dabei (4) neben Terpen und carboxylierten Produkte, β–Podocarprenoxycarbonsaure, β–Podocarprenketocarbonsäure, noch Diketo–α–Podocarpren erhalten wie folgende Schema:





(Forstchem. Institut, Kiushu Kaiserl. Univ.)

Ueber die Kohlenhydrate aus den Samen von *Phaseolus Mungo* (Lu-tou).

Von

Syozi MIKI

(Eingegangen June 30, 1931)

Die Gehalte an den Kohlenhydrate, angegeben in Prozenten der Trokensubstanz von Lu-tou, sind im folgenden:

1. Rohfaser	4.66%	4. Methylpentosan	Null
2. Pentosan	5.27%	5. Reduktionszucker	Null
3. Galaktan	1.03%	6. Totalle loesliche Kohlenhydrate (als Staerke)	66.02%

Loesliche Kohlenhydrate	1. Die in heissem 95%igem Alkohol loeslichen Kohlenhydrate (als Glukose)	2.02%
	2. Dextrin	3.40%
	3. Staerke	57.00%
	4. Hemicellulose und die anderen (als Galaktose)	3.03%

Ueber die in heissen 95%igem Alkohol loeslichen Kohlenhydrate.

Die mit Hilfe von Aether entfetteten, fein gepulverten Bohnen wurden mit heissem 95%igem Alkohol wiederholt ausgezogen. Die Beimischungen wurden mit Bleiessig, das Filtrat mit H_2S behandelt, dann wurden mit NH_3 neutralisiert und eingedunstet zum Sirup.

Durch die qualitativen Proben ist nachgewiesen worden, dass der dabei gewonnene Sirup, der Fehlingsche Loesung nicht reduziert, bei der Hydrolyse durch die verduennten anorganischen Saeure Glukose, Fruktose, Galaktose und Pentose (Xylose) lieferte. Dagegen liess sich keine Mannose nachweisen.

Isolierung und Nachweis der Raffinose und des Rohrzuckers.

a) Raffinose.

Der bei Zerlegung des Strontiumniederschlages, das aus der 80%igen Alkoholloesung des Sirups dargestellt wurde, erhaltene Sirup wurde mit Methylalkohol behandelt, um den darin enthaltenen Raffinose zu loesen. Aus der Methylalkoholloesung des Sirups konnte die Raffinose in Krystallen isoliert werden. Sie wurde durch Bestimmung der bei der Oxydation mit Salpetersaeure erhaltenen Schleimsaeuremenge (22.56%), ihres spezifischen Drehungsvermoegens ($[\alpha]_D^{20.5} = 104.8°$) und auch noch durch eine Krystallwasserbestimmung u. s. w. identifiziert.

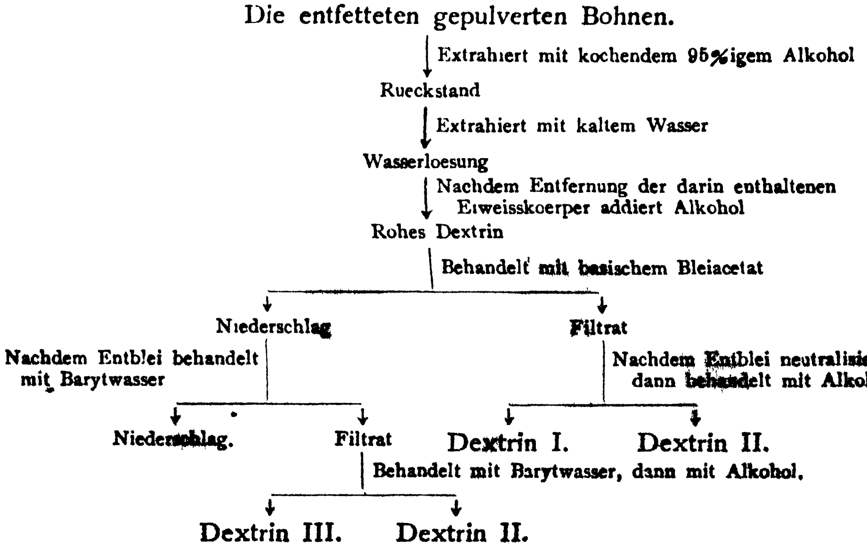
b) Rohrzucker.

Aus dem in Methylalkohol ungeloest gebliebenen Teile des Sirups konnte der Rohrzucker ohne Schwierigkeit in Krystallen gewonnen werden. Er hat die folgenden Eigenschaften: Monokline Krystalle. Schmelzpunkt 186°. $[\alpha]_D^{20.5} = 66.49°$.

Folglich ist wahrscheinlich, dass in den in heissem 95%igem Alkohol loeslichen Kohlenhydrate die Raffinose und der Rohrzucker vorhanden sind.

Ueber die in heissem 95%igem Alkohol unloelichen, in kaltem Wasser loeslichen Kohlenhydrate.

Nach folgendem Verfahren sind das Dextrin I, Dextrin II und Dextrin III isoliert worden.


Die entfetteten gepulverten Bohnen.
Extrahiert mit kochendem 95%igem Alkohol
Rueckstand
Extrahiert mit kaltem Wasser
Wasserloesung
Nachdem Entfernung der darin enthaltenen Eiweisskoerper addiert Alkohol
Rohes Dextrin
Behandelt mit basischem Bleiacetat
Niederschlag
Filtrat
Nachdem Entblei behandelt mit Barytwasser
Nachdem Entblei neutralisiert, dann behandelt mit Alkohol.
Niederschlag.
Filtrat
Dextrin I.
Dextrin II.
Behandelt mit Barytwasser, dann mit Alkohol.
Dextrin III.
Dextrin II.

Dextrin I.

Das Dextrin I ist nahezu identisch mit dem Amylodextrin von Lintner und Duell.

Einige Eigenschaften von Dextrin II sind aehnlich mit den des Erythrodextrins von Lintner und Duell, jedoch darauf man lieber das dabei gewonnene Dextrin II fuer ein Gemisch von Dextrin I und im folgenden erwaehntes Dextrin III halten.

Dextrin III.

Weisses, amorphes Pulver, loeslich in 70%igem Alkohol, unloeslich in 90%igem Alkohol. Jodreaktion rod. Reduktionsvermoegen Null. Bleiessig erzeugt in 5%iger Loesung einen Niederschlag; Barytwasser und Phosphowalframsaeure geben in 5%iger Loesung keine Faellung.

Staerke

Die Staerke von Lu–tou ist elliptisch (etwa 0.02∼0.03 mm. im Durchmesser) und enthaelt die folgenden Nichtkohlenhydrate:

Asche 0.14% : P_2O_5 0.0675% . SiO_2 0.0641% : Die assozierten Fettsaeuren 0.60%.

Ueber die Verzuckerung der Staerke von Lu–tou.

Die Resultate der Bestimmung des durch Verzuckerung von Lu–toustaerke, Weizenstaerke, Maisstaerke, Kartoffelstarke, Glutinoese Reistaerke, Gewoehnliche Reisstarke und Kaoliangstaerke mit Taka–Diastase entstandenen Reduktionszuckers sind in der nachfolgenden Tabelle zusammengestellt.

Diastase–Wirkung Stunden	1	3 5		20
Kartoffelstaerke	1.4352g	1.7524	1.9448	2.2841
Lu–toustaerke	1.2808	1.6873	1.8093	2.2598
Kaoliangstaerke (Huan Nien)	1.2808	1.6645	1.7821	2.2370
Kaoliangstaerke (Hei Ko)	1.2683	1.6417	1.7294	2.1661
Weizenstaerke	1.2683	1.5533	1.6351	2.1661
Maisstaerke	1.2568	1.5256	1.6121	2.1430
Glutinoese Reisstaerke	1.2568	1.5028	1.5891	2.1430
Gewoehnliche Reisstaerke	1.2455	1.4800	1.5659	2.1205

Wie man aus der Tabelle zu erschen vermag, ist die Verzuckerung von Lu–toustaerke mit Taka–Diastase besser als die anderen ausserdem Kartoffelstaerke.

Aber bei 3 stuendiger Verzuckerung der schon oben angegebenen verschiedenartigen Staerke mit 2.2%iger Salzsaeure im kochenden Wasserbade liefert Lu–toustaerke die groessere Glukosemenge als Glutinoese Reisstaerke, Maisstaerke und Kaoliangstaerke (Huan Nien) und die kleinere als die anderen.

Hemicellulosen.

Durch Anwendung von Norris und Preece–Methode (Bioch. J., **24**, 59, 1930), mittels welcher sie aus den Weizenkleien die verschiedenen Hemicellulosen herstellten, wurden die Hemicellulose I und Hemicellulose II aus

Lu-tou isoliert.

Hydrolyse der Hemicellulose I und Hemicellulose II mit Schwefelsaeure.

Sie geschah mit der 50-fachen Menge 3%iger Schwefelsaeure durch 4-stuendiges Erhitzen im kochenden Wasserbade und die mit Barytwasser entsaeuerten Fluessigkeiten wurden eingedampft zum Sirup.

Nachweis der Xylose in den Sirupe.

Aus den Sirupe der Hemicellulose I und Hemicellulose II wurden die Krystalle des Bertranschen Cadmiumbromxylonates erhalten, welches nach dem Umkrystallisieren Folgendes ergab.

$C_5H_9O_6CdBr+H_2O$	Ber.	Br	21.28,	Cd	29 94
I	Gef.	Br	21.35,	Cd	29.61
II	Gef.	Br	21 43,	Cd	29.70

Isolierung und Nachweis der Arabinose.

Durch Anwendung der von Maurenbrecher und Tollens (B., **39**, 3576, 1906) angegeben Methode der Trennung von Arabinose und Xylose auf diese Sirupe wurden die Krystalle des l-Arabinose-Diphenyl-Hydrazons (Schmelzp. 204°) erhalten. Durch die Zersetzung des dabei gewonnen Hydrazons mit Formaldehyd, wie Ruff und Ollendorf (B., **32**, 3236, 1899) angaben, ist Arabinose isoliert worden.

I $[\alpha]_D^{21^\circ} = +103.8^\circ$: II $[\alpha]_D^{21^\circ} = +104.7^\circ$

Nachweis der Galaktose.

Bei der Oxydation durch verduennte Salpetersaeure (S. G. 1.15) lieferten die beiden Sirupe Schleimsaeure (Schmelzp. 204°), welches Ca-Salz Folgendes ergab.

$CaC_6H_8O_8+1\,^1/_2\,H_2O$	Ber	CaO	20 38
I	Gef.	CaO	19 87
II	Gef	CaO	19 92

Die Proben auf den anderen Zuckerarten.

Die bei der Hydrolyse von Hemicellulose I und Hemicellulose II mit Schwefelsaeure erhaltenen beiden Sirupe gaben die Tollenssche Naphtoresorcin-Reaktion. Dagegen hatten die Versuche zur Gewinnung von Mannosephenylhydrazon aus den Sirupen keinen Erfolg und die Pinoff-Schiwanoffsche Probe auf Ketosen gleichfalls eine negative Resultate gab.

Die in den beiden Hemicellulosen vorhandenen Mengen von Pentose-, Galktose-, und Uronsaeure-Gruppe sind in folgender Weise bestimmt:

Hemicellulosen	I	II
Ausbeute an Furfurol	40.80%	36.52%
Ausbeute an CO_2	2.30%	3 85%
Uronsaeure Anhydrid ($CO_2 \times 4$)	9.20%	15.40%
Furfurol aus Uronsaeure-Gruppe	1.54%	2 57%
Furfurol aus Pentose-Gruppe	39.26%	33.95%

Chemische Untersuchungen über das Glukomannan aus "Konjak".

V. Mitteilung. Methylierung des Glukomannans.

Von

Kitsuji NISHIDA und Hideo HASHIMA

(Eingegangen am 26 Juni 1931)

Der Methylierungsversuch gibt die Aufklärung über das Konstitutionsproblem des Glukomannans und durch ihn wird ein gewisser eindeutiger Abschluss erreicht. Zunächst wurden 10 g. Glukomannan mit 200 ccm. 15%-iger Natronlauge und 96 g. Dimethylsulfat methyliert. Nach 6-mal Methylierung betrug der Methoxylgehalt 34 %. Durch weitere Methylierungen, unter Verwendung von 20 g. Glukomannan, 30 ccm. 30%iger Natronlauge und 288 g. Dimethylsulfat für jeden Methylierungsversuch, war dann der Methoxylgehalt ein höhere geworden und 12～14 malige Methylierung diesen Wert 41.5% hatte erreichen lassen.

Bei der Methylierung von 2 g. methylierten Glukomannans (41.6% Methoxyl) mit 50 ccm. Methyljodid und 36 g. Silberoxyd erreichte der Wert nicht über 41.6% OCH_3. Wir müssen hieraus den Schluss ziehen, dass das Glukomannan bei der Methylierung mit Dimethylsulfat und Alkali oder mit Methyljodid und Silberoxyd gleichviel Methoxyl liefert wie die Cellulose und das Lichenin, d. h. dass er nicht über 42% zunehmen kann, und dass die Methylierung beim Arbeiten mit 30%iger Natronlauge und bei niedrigerer Temperatur leichter ist.

Das Methylglukomannan (OCH_3 41.6%) ist in kaltem Wasser kolloidal lösbar, in heissem Wasser dagegen tritt wieder Ausflockung ein, wie der Methyläther der Cellulose, des Lichenin und der Starke. Es löst sich in Chloroform, Bromoform, Alkohol, Aceton, Eisessig und Essigester, doch haben wir eine Molekulargewichtsbestimmung nicht ausgeführt.

Die Hydrolyse des Glukomannanathers führt zu methylierten Hexosen, und hat keine Abspaltung von Methylgruppen im Gefolge. Zu diesem Zweck wurden 29.8517 g. methyliertes Glukomannan (OCH_3 41.5 %) mit 1%iger methylalkoholischer Salzsäure in Bonbenröhren zu den methylierten Methylglukosiden und -mannosiden aufgespalten. Das Spaltungsprodukt wurde mit Silberkarbonat neutralisiert und es wurden nach dem Trocknen im Hochvakuum bei der fraktionierten Destillation fünf Fraktionen aufgefangen. Die ersten drei Fraktionen zeigten ein fast farbloses, die späteren ein schwach gelbliches Aussehen, und alle färbten beim Stehen in langer Zeit nach. Die Analyse ergaben.

	Sdp. (°C)	OCH_3 %		n_D^{15}	$[\alpha]_D^{20}$ in Wasser	$[\alpha]_D^{20}$ in Methylalkohol
I Fraktion	124~125	50.83	50.11	—	—	—
II. Fraktion	125~127	51.10	50.74	1.4604	+43.8°	+45.6°
III. Fraktion	127~129	48.68	48.11	1.4587	+42.3°	+52.8°
IV-A. Fraktion	126~128	47.96	48.57	1 4597	+43.0° +42.5°	—
IV-B. Fraktion	128~139	47.16	47.08	1.4626	+45.9°	+56.6°

Aus diesen Versuchen geht also hervor, dass die Konstanten der Fraktionen, die aus methyliertem Glukomannan einerseits, aus Methylcellulose und -lichenin anderseits durch Spaltung gewonnen worden sind, eine voreinander erheblich abweichende Zusammensetzung zu haben scheinen, da das Glukomannan aus 2 Mol Mannose und 1 Mol Glukose besteht und die Spaltprodukte müssen Trimethylmannosid und -glukosid enthalten.

Die I. und II. Fraktion stehen im Methoxylgehalt ca. 1.1% unter denjenigen, welche sich für ein Trimethylmethylmannosid oder -glukosid aussprechen lassen wurden (52.6%); es muss darin kleine Mengen Dimethylmethylmannosid oder -glukosid (41.8%), das in der III.-IV. Fraktion überwiegt, enthalten sein. Der Methoxylgehalt der II. Fraktion war sehr gleichmässig, aber Brechungsindex war etwas hoch, und die spezifische Drehung war klein, verglichen nicht mit denjenigen, die bei Methylcellulose und -lichenin durch Abbau erhalten werden.

Die II. Fraktion wurde mit wasseriger Salzsaure verseift und dabei schied sich nicht die kristallisierte 2, 3, 6-Trimethylglukose (41.89% OCH_3) nach langem Stehen wie diejenige, die aus der ersten Fraktion von Methylbaumwolle und Methylolichenin abgebaut wurde, ab. Dieser Sirup erwies:

%OCH_3 40.49 ; 40.49.

Es muss darin eine Spur Dimethylhexose (29.81% OCH_3) enthalten sein. Wir haben diesen Sirup mit Salpetersäure (spez. Gew. 1.20) oxydiert und erhielten Trimethylzuckersaurelakton oder -mannozuckersaurelakton mit folgenden Eigenschaften:

OCH_3-Gehalt gef.	37.15; 36.89%
Berechnet für Trimethylzuckersäurelakton	39.74%
Verbrauch von N/10-Natronlauge.	10.00 ccm. (Substanz 0.116 g.)

Aus diesem Ergebnis geht also hervor, dass die I. und II. Fraktion 2, 3, 4-Trimethylhexosid waren mit kleine Mengen Dimethylhexosid und nicht 2, 3, 6-Trimethylmethylhexosid, und mit grosser Wahrscheinlichkeit handelte es sich um 2, 3, 4-Trimethylglukosid und nicht Mannosid. Die III, IV-A und -B Fraktion wurden auch wie die erste Fraktion mit Salzsäure verseift, dabei wir Trimethylhexose erhielten, die nach langem Stehen keine Kristalle abschied. Die Analyse ergab:

Fraktion	%OCH_3		$[\alpha]_D^{20}$
III	38.04	38.01	+36° in Wasser
IV–A	38.73	38.46	+18.5° in Chloroform
IV–B	36.26	36 38	+11.6° in Chloroform

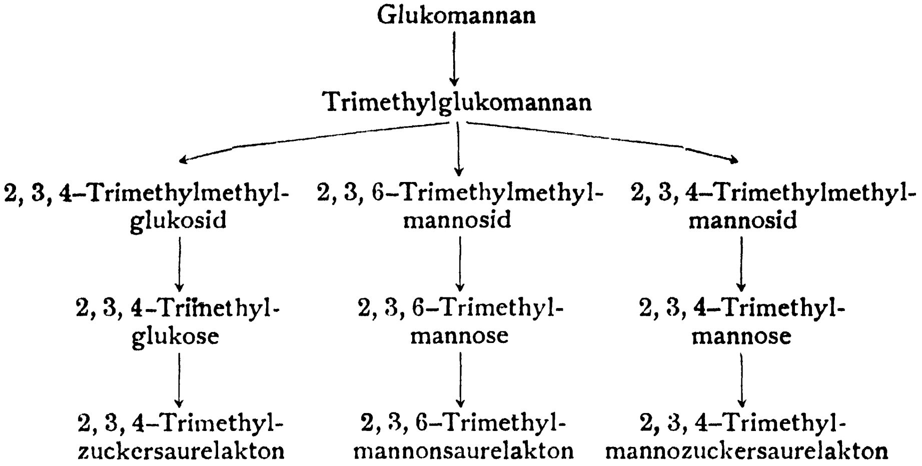
Im Methoxylgehalt stehen diese Fraktionen ca. 3,3～5.6% unter der Trimethylhexose; es muss darin bereits geringe Mengen Dimethylhexose enthalten sein. Dies Fraktion wurden wie oben mit Salpetersaure oxydiert, und dabei wurden Mono– und Dikarbonsaurelakton mit folgenden Eigenschaften gewonnen.

Aus der III.	Fraktion	OCH_3–Gehalt gef	36.13;	35 78
Aus der IV–A	Fraktion	OCH_3–Gehalt gef.	35 18;	35.28
Aus der IV–B	Fraktion	OCH_3–Gehalt gef	33.89;	33.76

Fraktion	Substanz	Verbrauch von N/10-Natronlauge	Fraktion	Substanz	Verbrauch von N/10-Natronlauge
III. Fraktion	0 1071 g	7 52ccm	IV–B Fraktion	0.1180	9 16
IV–A Fraktion	0.1762	9.98	IV–B Fraktion	0.2631	19 33

Aus hier nahm in der III. und der IV–B Fraktion die Aciditat zu, wahrend der Sauregehalt in der IV–A Fraktion zur Halfte zurückging. Bei all diesem Saurelakton handelte es sich selbstverstandlich um Mischungen, aus denen die erste (aus der III Fraktion) eine Losung von Trimethylzuckersaurelakton mit kleinen Mengen Trimethylmannonsaurelakton, die zweite (aus der IV–A Fraktion) ein Sirup von Trimethylmannonsaurelakton mit einer Spur Tri– und Dimethylmannozuckersürelakton, die dritte (aus der IV–B Fraktion) eine Mischung von Tri– und Dimethylmannozuckersaurelakton waren. Bei 2, 3, 6–Trimethylmethylglukosid Fraktion finden sich nach Verseifung mit Salzsaure bekanntlich Kristalle von 2, 3, 6–Trimethylglukose, die mit Salpetersaure zu 2, 3, 6–Trimethylglukonsaurelakton (Monokarbonsaurelakton) oxydieren müssen. Der grossere Teil aus der IV–A Fraktion war 2, 3, 6–Trimethylmethylhexosid, aber seine Eigenschaften und das Ergebnis ihrer Analyse zeigte nicht so grosse Aehnlichkeit, dass es sich hier um 2, 3, 6–Trimethylmethylglukosid handeln konnte; der Versuch, in welchem aus methyliertem Glukomannan durch Spaltung keine Kristalle gewonnen wurden, bildete also auch eine Stütze für die Abwesenheit der 2, 3, 6-Trimethylglukose. Da die Trimethylglukose und –mannose mit Phenylhydrazin kein Phenylosazon liefert, so muss am Kohlenstoffatom 2 eine Methoxylgruppe sitzen. Als Gesamtergebnis der im vorstehenden skizzierten Untersuchungen auf dem

Gebiete des Methylglukomannans folgt, dass das Spaltprodukt auf die Weise gewonnen wurde, wie sie das nachstehende Schema zeigt:


Glukomannan
Trimethylglukomannan
2, 3, 4-Trimethylmethylglukosid
2, 3, 6-Trimethylmethylmannosid
2, 3, 4-Trimethylmethylmannosid
2, 3, 4-Trimethylglukose
2, 3, 6-Trimethylmannose
2, 3, 4-Trimethylmannose
2, 3, 4-Trimethylzuckersaurelakton
2, 3, 6-Trimethylmannonsaurelakton
2, 3, 4-Trimethylmannozuckersaurelakton

(Forstchem Institut, Kiushu Kaiserl. Univ)

Untersuchung über das ätherische Oel aus *Podocarpus macrophylla Don.* III.

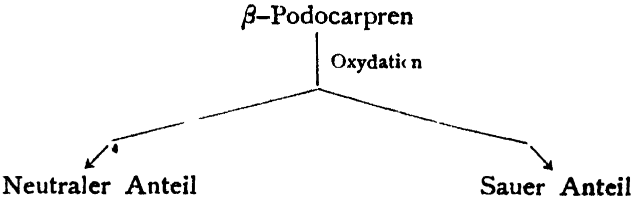
Mitteilung. Ueber die oxydierenden Produkten der neuen Diterpene (α– und β–Podocarpren).

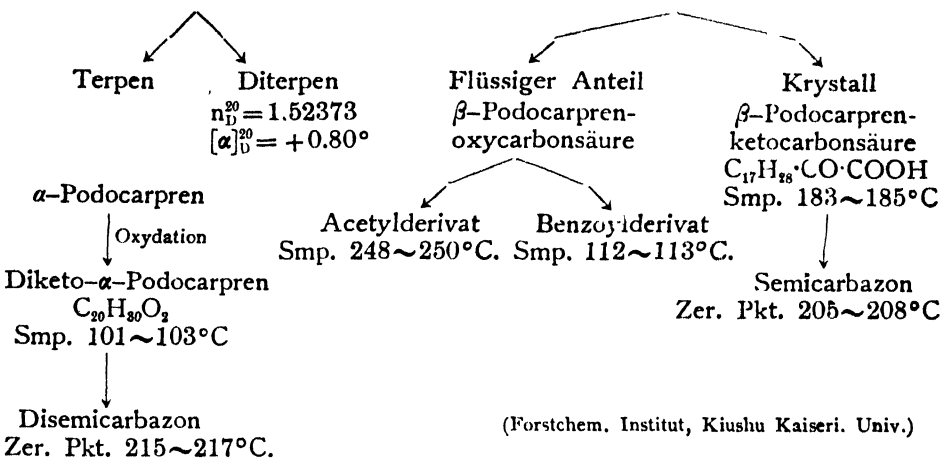
Von

Kitsuji NISHIDA und Hidetaka UOTA

(Eingegangen am 26 Juni 1931)

Zur Konstitutionsaufklarung des α– und β–Podocarpren führten wir den oxdierenden Abbau der Diterpene (1) mit Kaliumpermanganatlosung (2) mit Braunstein und Schwefelsaure (3) mit Kaliumbichromat und Schwefelsaure (4) mit Kaliumpermaganatlosung bei Gegenwart von Alkali durch. Es wurde dabei (4) neben Terpen und carboxylierten Produkte, β–Podocarprenoxycarbonsaure, β–Podocarprenketocarbonsaure, noch Diketo–α–Podocarpren erhalten wie folgende Schema:





(Forstchem. Institut, Kiushu Kaiseri. Univ.)

Ueber die Kohlenhydrate aus den Samen von *Phaseolus Mungo* (Lu-tou).

Von

Syozi Miki

(Eingegangen June 30, 1931)

Die Gehalte an den Kohlenhydrate, angegeben in Prozenten der Trokensubstanz von Lu-tou, sind im folgenden:

1. Rohfaser	4.66%	4. Methylpentosan	Null
2. Pentosan	5.27%	5. Reduktionszucker	Null
3. Galaktan	1.03%	6. Totalle loesliche Kohlenhydrate (als Staerke)	66.02%

Loesliche Kohlenhydrate	1. Die in heissem 95%igem Alkohol loeslichen Kohlenhydrate (als Glukose)	2.02%
	2. Dextrin	3.40%
	3. Staerke	57.00%
	4. Hemicellulose und die anderen (als Galaktose)	3.03%

Ueber die in heissen 95%igem Alkohol loeslichen Kohlenhydrate.

Die mit Hilfe von Aether entfetteten, fein gepulverten Bohnen wurden mit heissem 95%igem Alkohol wiederholt ausgezogen. Die Beimischungen wurden mit Bleiessig, das Filtrat mit H_2S behandelt, dann wurden mit NH_3 neutralisiert und eingedunstet zum Sirup.

Durch die qualitativen Proben ist nachgewiesen worden, dass der dabei gewonnene Sirup, der Fehlingsche Loesung nicht reduziert, bei der Hydrolyse durch die verduennten anorganischen Saeure Glukose, Fruktose, Galaktose und Pentose (Xylose) lieferte. Dagegen liess sich keine Mannose nachweisen.

Isolierung und Nachweis der Raffinose und des Rohrzuckers.

a) Raffinose.

Der bei Zerlegung des Strontiumniederschlages, das aus der 80%igen Alkoholloesung des Sirups dargestellt wurde, erhaltene Sirup wurde mit Methylalkohol behandelt, um den darin enthaltenen Raffinose zu loesen. Aus der Methylalkoholloesung des Sirups konnte die Raffinose in Krystallen isoliert werden. Sie wurde durch Bestimmung der bei der Oxydation mit Salpetersaeure erhaltenen Schleimsaeuremenge (22.56%), ihres spezifischen Drehungsvermoegens ($[\alpha]_D^{20.5}=104.8°$) und auch noch durch eine Krystallwasserbestimmung u. s. w. identifiziert.

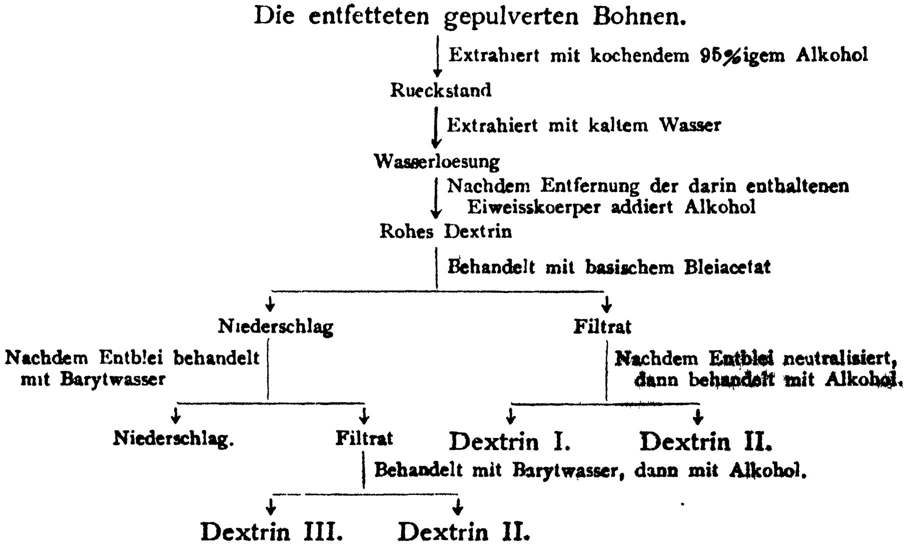
b) Rohrzucker.

Aus dem in Methylalkohol ungeloest gebliebenen Teile des Sirups konnte der Rohrzucker ohne Schwierigkeit in Krystallen gewonnen werden. Er hat die folgenden Eigenschaften: Monokline Krystalle. Schmelzpunkt 186°. $[\alpha]_D^{20.5}=66.49°$.

Folglich ist wahrscheinlich, dass in den in heissem 95%igem Alkohol loeslichen Kohlenhydrate die Raffinose und der Rohrzucker vorhanden sind.

Ueber die in heissem 95%igem Alkohol unloelichen, in kaltem Wasser loeslichen Kohlenhydrate.

Nach folgendem Verfahren sind das Dextrin I, Dextrin II und Dextrin III isoliert worden.


Die entfetteten gepulverten Bohnen.
Extrahiert mit kochendem 95%igem Alkohol
Rueckstand
Extrahiert mit kaltem Wasser
Wasserloesung
Nachdem Entfernung der darin enthaltenen Eiweisskoerper addiert Alkohol
Rohes Dextrin
Behandelt mit basischem Bleiacetat
Niederschlag
Filtrat
Nachdem Entblei behandelt mit Barytwasser
Nachdem Entblei neutralisiert, dann behandelt mit Alkohol.
Niederschlag.
Filtrat
Dextrin I.
Dextrin II.
Behandelt mit Barytwasser, dann mit Alkohol.
Dextrin III.
Dextrin II.

Dextrin I.

Das Dextrin I ist nahezu identisch mit dem Amylodextrin von Lintner und Duell.

Einige Eigenschaften von Dextrin II sind aehnlich mit den des Erythrodextrins von Lintner und Duell, jedoch darauf man lieber das dabei gewonnene Dextrin II fuer ein Gemisch von Dextrin I und im folgenden erwaehntes Dextrin III halten.

Dextrin III.

Weisses, amorphes Pulver, loeslich in 70%igem Alkohol, unloeslich in 90%igem Alkohol. Jodreaktion rod. Reduktionsvermoegen Null. Bleiessig erzeugt in 5%iger Loesung einen Niederschlag; Barytwasser und Phosphowalframsaeure geben in 5%iger Loesung keine Faellung.

Staerke

Die Staerke von Lu–tou ist elliptisch (etwa 0.02～0.03 mm. im Durchmesser) und enthaelt die folgenden Nichtkohlenhydrate:

Asche 0.14% : P_2O_5 0.0675% : SiO_2 0.0641% : Die assozierten Fettsaeuren 0.60%.

Ueber die Verzuckerung der Staerke von Lu–tou.

Die Resultate der Bestimmung des durch Verzuckerung von Lu–toustaerke, Weizenstaerke, Maisstaerke, Kartoffelstarke, Glutinoese Reistaerke, Gewoehnliche Reisstarke und Kaoliangstaerke mit Taka–Diastase entstandenen Reduktionszuckers sind in der nachfolgenden Tabelle zusammengestellt.

Diastase-Wirkung Stunden	1	3.5	5	20
Kartoffelstaerke	1.4352g	1.7524	1.9448	2.2841
Lu–toustaerke	1.2808	1.6873	1 8093	2.2598
Kaoliangstaerke (Huan Nien)	1.2808	1.6645	1.7821	2.2370
Kaoliangstaerke (Hei Ko)	1.2683	1.6417	1.7294	2.1661
Weizenstaerke	1.2683	1.5533	1.6351	2 1661
Maisstaerke	1.2568	1.5256	1.6121	2.1430
Glutinoese Reisstaerke	1.2568	1.5028	1.5891	2 1430
Gewoehnliche Reisstaerke	1.2455	1.4800	1.5659	2.1205

Wie man aus der Tabelle zu ersehen vermag, ist die Verzuckerung von Lu–toustaerke mit Taka–Diastase besser als die anderen ausserdem Kartoffelstaerke.

Aber bei 3 stuendiger Verzuckerung der schon oben angegebenen verschiedenartigen Staerke mit 2.2%iger Salzsaeure im kochenden Wasserbade liefert Lu–toustaerke die groessere Glukosemenge als Glutinoese Reisstaerke, Maisstaerke und Kaoliangstaerke (Huan Nien) und die kleinere als die anderen.

Hemicellulosen.

Durch Anwendung von Norris und Preece–Methode (Bioch. J., **24**, 59, 1930), mittels welcher sie aus den Weizenkleien die verschiedenen Hemicellulosen herstellten, wurden die Hemicellulose I und Hemicellulose II aus

Lu–tou isoliert.

Hydrolyse der Hemicellulose I und Hemicellulose II mit Schwefelsaeure.

Sie geschah mit der 50–fachen Menge 3%iger Schwefelsaeure durch 4–stuendiges Erhitzen im kochenden Wasserbade und die mit Barytwasser entsaeuerten Fluessigkeiten wurden eingedampft zum Sirup.

Nachweis der Xylose in den Sirupe.

Aus den Sirupe der Hemicellulose I und Hemicellulose II wurden die Krystalle des Bertranschen Cadmiumbromxylonates erhalten, welches nach dem Umkrystallisieren Folgendes ergab.

$C_5H_9O_6CdBr+H_2O$	Ber.	Br	21.28,	Cd	29.94
I	Gef.	Br	21.35,	Cd	29.61
II	Gef.	Br	21.43,	Cd	29.70

Isolierung und Nachweis der Arabinose.

Durch Anwendung der von Maurenbrecher und Tollens (B., **39**, 3576, 1906) angegeben Methode der Trennung von Arabinose und Xylose auf diese Sirupe wurden die Krystalle des I–Arabinose–Diphenyl–Hydrazons (Schmelzp. 204°) erhalten. Durch die Zersetzung des dabei gewonnen Hydrazons mit Formaldehyd, wie Ruff und Ollendorf (B., **32**, 3236, 1899) angaben, ist Arabinose isoliert worden.

I $[\alpha]_D^{21°} = +103.8°$: II $[\alpha]_D^{21°} = +104.7°$

Nachweis der Galaktose.

Bei der Oxydation durch verduennte Salpetersaeure (S. G. 1.15) lieferten die beiden Sirupe Schleimsaeure (Schmelzp. 204°), welches Ca–Salz Folgendes ergab.

$CaC_6H_8O_8+1\,{}^1/_2\,H_2O$	Ber	CaO	20.38
I	Gef.	CaO	19.87
II	Gef.	CaO	19 92

Die Proben auf den anderen Zuckerarten.

Die bei der Hydrolyse von Hemicellulose I und Hemicellulose II mit Schwefelsaeure erhaltenen beiden Sirupe gaben die Tollenssche Naphtoresorcin–Reaktion. Dagegen hatten die Versuche zur Gewinnung von Mannosephenylhydrazon aus den Sirupen keinen Erfolg und die Pinoff–Schiwanoffsche Probe auf Ketosen gleichfalls eine negative Resultate gab.

Die in den beiden Hemicellulosen vorhandenen Mengen von Pentose–, Galktose–, und Uronsaeure–Gruppe sind in folgender Weise bestimmt:

Hemicellulosen	I	II
Ausbeute an Furfurol	40.80%	36.52%
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Furfurol aus Pentose–Gruppe	39.26%	33.95%

Then the maximum value of x is about 18 by graphical solution (Fig. 6 and Table VIII).

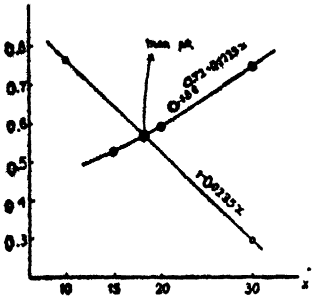

Fig. 6

Table VIII.

x	$1-0.0235x$	$0.18e^{0.72+0.0235x}$
10	0.765	—
15	0.644_5	0.525
20	0.530	0.593
30	0.295	0.747

Conclusion

Dietary protein (for use in trout fry culture) can be utilized for growth to 42,5% and about 18% of it, in case of dietary or supplied body or assimilated protein are of the same value, would be at an economical loss.

Summary

A coefficient of biological value of utilization (A) and an amount (x%) of dietary protein are in the relation as the following equation:

$$A = e^{-0.72-0.0235x}$$

A value of A is, therefore, gradually decreased with increasing of x.

Ax indicates a value of real utilization of dietary protein and it shows the maximum value when $x = 42.5_5$%.

Then, although the value of utilization (Ax) should bė ascended until $x =$ 42.5, a ratio of increase of Ax to that of x is not equal at every point, i. e. values of

$$\frac{Ax}{(Ax)_{\max}} - \frac{ax}{(Ax)_{\max}} \quad \text{or} \quad \frac{Ax}{7.67} - \frac{x}{42.5_5}$$

increase, at first, and then descend to zero on that point, and the maximum point of these values is in the case of $x \doteqdot 18$.

The author designated that point "Economical Maximum Point of Utilization of Dietary Protein".

From the results, an economical protein content of an artificial diet use in feeding trout-fry may be 20%~30%.

In conclusion I desire to express my thanks to Mr. Sato and Mr. Hoshino for their heepful abvice in this work.

Digestion Experiment of Soy Bean Cake and Kaoliang with Poultry.

By

Kozo Suzuki

(*Imperial Zootechnical Experiment Station, Chiba*)

(Received September 25, 1931)

1. Digestion Experiment of Soy Bean Cake.

Experimental Procedure.

The digestion experiment of soy bean cake was undertaken with two 2-years-old single comb white leghorn cocks. After a twenty-four hours' fast, they were dosed with 1 g. of thymol and a couple of hours later were given 10 c.c. of castol oil to remove the parasites in their intestines. Three days later, the birds were starved again for twenty-four hours and were performed the operation to produce an artificial anus by cutting the intestine at a point just back of where the urine emptied into it, and bringing the end out to the abdomen walls. After the operation, the health of the birds was carefully watched. When the operated parts were completely heald up, the cocks were fed with the basal ration, in which was mixed a small amount of soy bean cake ground fine enough to pass through a sieve with pores of 2.5 mm. in diameter. Day by day, the amount of the ground soy bean cake was increased and after five days the whole ration was changed to ground soy bean cake alone and for a few days more the birds were compelled to eat this food only. After a while the birds became accustomed to the apparatus of rubber bags which were held in place for collecting the urine and dung separately.

Before the collection of the dung was begun, the cocks were starved for fifteen hours, during which time they excreted all the dung in the intestines in first few hours. At the end of the fifteen hours past they were compelled to swallow 0.5 g. of charcoal which was suspended in about 10 c.c. of water. After four or five hours the birds excreted the charcoal alone. After a fifteen hours' starvation, a measured amount of ground soy bean cake was supplied in a vessel.

Twenty-four hours later, the food-vessel was taken away and the amount of food which remained was weighed to know the quantity of food consumption of twenty-four hours. Then, after four hours the birds were compelled again to swallow 0.5g. of charcoal suspended in about 10c.c. of water. After four or five hours the charcoal was excreted alone.

The amount of dung which was excreted in the time between the end of the first excretion of charcoal and the begining of its second excretion represented exactly the undigested part of soy bean cake which has been taken in the experimental period of twenty-four hours.

Bird A took 139.0 g. of soy bean cake, and bird B 154.8 g. in the experimental period of twenty-four hours; bird A has excreted 247.2 g. of wet dung, and bird B 275.0 g.

Composition of Soy Bean Cake and the Dung.

		Moisture	Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Crude ash	Pure protein
		%	%	%	%	%	%	%	%
Air-dried state	Soy bean cake	13.640	80.860	43.563	7.298	3 950	26.049	5.500	42.440
	Dung of bird A	12 790	76.460	19.760	1 653	11.188	43.859	10.750	10.779
	Dung of bird B	12 575	76.335	18.189	1.800	11.845	44.501	11.090	11.901
Moisture-free state	Soy bean cake		93.631	50.443	8.451	4 574	30.163	6.369	49.143
	Dung of bird A		87.673	22.658	1.895	12 829	50.291	12.327	12.360
	Dung of bird B		87.315	20.805	2 059	13.549	50 902	12.685	13.613

Calculation of Digestibility Coefficient.

The 139.0 and 154.8 g. of soy bean cake which were taken by both birds can be converted into 120.0 and 133.7 g. of the moisture-free state, and 247.2 and 275.0 g. of wet dung were calculated to 43.0 and 46.3 g. of the moisture-free state respectively. Therefore, the digestibility coefficient of soy bean cake can be obtained as follows:

			Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Pure protein
Bird A	In dried soy bean cake 120.0 g.	(g)	112.357	60.532	10.141	5.830	35.856	58.847
	In dried dung 43.0 g.	(g)	37.699	9.743	0.815	5.637	21.504	5.266
	Digested quantity	(g.)	74.658	50.789	9 326	0.193	14 852	53.581
	Digestibility coefficient	(%)	66.447	83 904	91.963	3.310	40.027	91.061
Bird B	In dried soy bean cake 133.7 g.	(g.)	125.185	67.442	11.299	6.479	39.950	65.565
	In dried dung 46.3 g.	(g)	40.427	9 633	0.953	6.403	23.438	6.255
	Digested quantity	(g)	84.758	57.809	10.346	0 076	16.512	59 310
	Digestibility coefficient	(%)	67.652	85.717	91.566	1.173	41.382	90.460

Average Digestibility Coefficient of Soy Bean Cake.

Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Pure protein
67.0%	84.8%	91.8%	2.2%	40.7%	90.7%

2. Digestion Experiment of Kaoliang.

The digestion experiment of ground kaoliang was carried out with the same cock (Bird A) and with the same procedure as the above mentioned experiment of the soy bean cake.

The cock took 231.0 g. of ground kaoliang in the experimental period of twenty-four hours and excreted 221.0 g. of wet dung.

Composition of Kaoliang and the Dung.

		Moisture	Organic matter	Curde protein	Crude fat	Crude fiber	Nitrogen-free extract	Crude ash	Pure protein
Kaoliang	Air-dried state	% 12.425	% 85.425	% 11 228	% 3.590	% 1.340	% 69.262	% 2.155	% 10.689
	Dried state		97.539	12 821	4.099	1.530	79.089	2.461	12.206
Dung	Air-dried state	14.540	79.280	32.694	4.714	5.145	36.727	6.180	29.102
	Dried state		92 769	38.256	5.521	6.020	42.976	7.231	34.053

Culculation of Digestibility Coefficient.

The 231.0 g. of kaoliang can be converted into 202.3 g. of the moisture-free state and 221.0 g. of wet dung were calculated to 52.6 g. of the moisture-free state.

Digestibility coefficient of kaoliang is obtained as follows:

		Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Pure protein
In dried kaoliang 221.0 g.	(g.)	197.321	25.937	8.292	3.095	159.997	24.693
In dried dung 52.6 g.	(g)	48.796	20.123	2.904	3 166	22 605	17.911
Digested quantity	(g.)	148.525	5.814	5 388	(−)0.071	137.392	6.782
Digestibility coefficient	(%)	75 3	22.4	65 0	—	85.9	27 5

Digestibility Coefficient of Kaoliang.

Organic matter	Crude protein	Crude fat	Nitrogen-free extract	Pure protein
75.3%	22 4%	65.0%	85.9%	27.5%

On the Identification of Phosphate-compounds in Soil.

By

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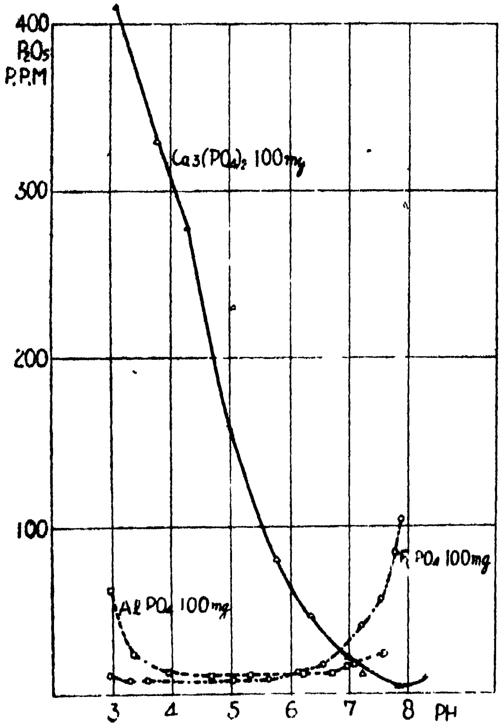
1. In order to identify soil-phosphates qualitatively and quantitatively by determining their solubility in solution with various pH, the writer tested at first the solubility of pure tricalcium phosphate, ferric phosphate and aluminium phosphate and obtained the following result (shown in the figure) which

shows that;

a. Tricalcium phosphate has minimum solubility at pH 7.68 which increases rapidly at more acid and a little at more alkaline side.

b. Aluminium phosphate dissolves least at pH between 4.07 and 6.93 and the solubility increases slowly both at more acid and alkaline sides.

c. Ferric phosphate shows minimum solubility at pH between 3 and 6 and it dissolves markedly at more alkaline side.



The characteristic of the above solubility curves was not changed by the amount of phosphate applied, by the co-existence of the above two or three salts and small quantity of soluble phosphate, although in the last case, larger solubility was noted at alkaline side for $Ca_3(PO_4)_2$, at acid side for $FePO_4$ and at all range of reaction for $AlPO_4$.

2. The writer made the same experiment with 11 soils and identified their phosphates by comparing the solubility curve with pure phosphates with the following result.

	phosphate identified	ratio of phosphate
4 soils	$FePO_4$	
2 soils	$Ca_3(PO_4)_2$ and $FePO_4$	1 : 1～1 : 2
3 soils	〃 〃	1 : 10～1 : 20
1 soil	$AlPO_4$ and $FePO_4$	1 : 10
1 soil	Same as above but with very small quantity of $Ca_3(PO_4)_2$	

3. It was also experimented that the change of monocalcium phosphate after various standing with soil, can be traced with the method.